EFFECTS OF CHRONIC OMEGA-3 FATTY ACID SUPPLEMENTATION ON ERYTHROCYTE DEFORMABILITY AND MUSCLE MICROVASCULAR OXYGENATION IN ENDURANCE TRAINED CYCLISTS

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ABSTRACT

The ability to perform endurance exercise is dependent on the transport of oxygen to the active skeletal muscles. For oxygen to perfuse into the skeletal muscle, flexibility of red blood cells is crucial in determining the passage through narrow capillaries, thus the erythrocytes must deform to increase regional blood flow through the microvasculature. It has been shown at submaximal exercise and in conditions of acute hypoxia, erythrocyte deformability is decreased, however supplementation of omega-3 polyunsaturated fatty acids (PUFAs) has been shown to increase erythrocyte deformability. **PURPOSE:** The purpose of this study is to determine the effects of chronic omega-3 fatty acid supplementation on erythrocyte deformability and tissue oxygenation in highly trained cyclists during submaximal exercise in both normoxic and hypoxic conditions. **METHODS:** The protocol was performed on thirteen highly trained cyclists (VO_2 max ≥55ml/kg/min). The subjects were divided into treatment (2g of DHA, 3g of EPA, and 100mg of Vitamin E) and placebo (safflower oil) groups. The subjects' visits pre- and post-supplementation followed identical protocols with one visit occurring in normoxia and the other in acute hypoxia (FIO_2 =15%). Each visit involved 2 submaximal cycling bouts of 3 minutes, equaling 25% and 50% of peak power, and one bout at 75% of peak power to exhaustion. Erythrocyte elongation index (EI) and near infrared spectroscopy (NIRS) measures were recorded for each trial. **RESULTS:** There were no significant differences in Elongation Index (EI) when comparing baseline values to measurements taken post-exercise in the hypoxic condition prior to supplementation. After supplementation, there were no significant differences in baseline EI when compared to pre-supplementation values. After the hypoxic exercise trial, EI at 20 Pa of shear stress was significantly greater within the PUFA group post-supplementation (pre: 0.574 ± 0.004; post: 0.580 ± 0.003; $p<0.05$).EI at 20 Pa was significantly greater post-supplementation in the PUFA group compared to the placebo group (PUFA: 0.580 ± 0.003; placebo: 0.574 ± 0.006; $p<0.05$).There were no significant difference in any of the NIRS measures any common workload **CONCLUSION:** Erythrocye deformability did not decrease with exercise in hypoxia, nor did baseline levels change with omega-3 supplementation, and only a marginal significant difference on post-exercise erythrocyte deformability measures was demonstrated. There was no effect on microvascular tissue oxygenation measures.

TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION

The ability to perform endurance exercise is dependent on the transport of oxygen through the vasculature and the perfusion of oxygen into the active skeletal muscles. This relies on the capability of the oxygen-carrying erythrocytes to reach the appropriate tissues. As regional blood flow increases, blood perfuses into the microvasculature, which can be as narrow as 3 μm in diameter[1]. Since erythrocytes are, on average, 7μm in diameter, the tissues supplied by the microvasculature rely on the ability of erythrocytes to deform for the delivery of oxygen [2]. In cases where the erythrocytes are unable to adequately deform, they bypass the most narrow vessels of the microvasculature [3], thus causing areas of skeletal muscle to lack adequate oxygenation. There are a variety of conditions where erythrocyte deformability is reduced, and there are a number of factors that influence these changes. It has been suggested that decreased intracellular ATP, as well as decreased arterial hemoglobin saturation could decrease deformability [4, 5] such as during submaximal exercise [3] and at altitude [6]. In these cases, the delivery of oxygen to active tissues may be limited, and (if so) consequently the ability to perform endurance exercise would be reduced.

In the cases of submaximal exercise and acute hypoxia, blood flow is increased to regions of active tissue [3], however the decrease of erythrocyte deformability at these same conditions may prevent the maximal effects of these adaptations. In order for maximal oxygen delivery to occur in the active skeletal muscle, erythrocyte deformability must be maintained to allow for passage through the microvasculature. Changes occurring in erythrocyte deformability have been investigated in the effects of various diseases states[1, 7], drugs, varying levels of physical activity[8], and environmental conditions[3, 9-11]. It has been shown that erythrocyte

deformability is higher in elite athletes than untrained controls [8], however the research exploring the effect of erythrocyte deformability on microvascular oxygen delivery in highly trained individuals has not been extensively pursued.

Purpose of the study

There are three factors that influence whether erythrocytes maintain adequate deformability for transport through the microvasculature: 1) the biconcave shape, resulting in the high surface area-to-volume ratio of the cell, 2) the viscoelastic properties of the cell membrane, and 3) the viscosity of the hemoglobin solution within the cell [1]. In regards to the viscoelastic properties of the cell membrane, a correlation has been demonstrated in the literature between erythrocyte deformability and phospholipid content in the cell membrane[12]. This suggests that by increasing the phospholipid content in the lipid bilayer of the red cell membrane, erythrocyte deformability could be improved, and therefore oxygenation of tissues supplied by the microvasculature could increase.

Omega-3 polyunsaturated fatty acids (PUFAs) are a phospholipid whose clinical properties have been investigated extensively[1]. It has been demonstrated that chronic supplementation of PUFAs increases erythrocyte deformability and skeletal muscle blood flow [13]. However, whether any omega-3 mediated increase in erythrocyte deformability leads to improvements in microvascular oxygenation during exercise and/or hypoxic exposure is not known. The purpose of this study is to determine the effects of chronic omega-3 fatty acid supplementation on erythrocyte deformability and tissue oxygenation in highly trained cyclists during submaximal exercise in both normoxic and hypoxic conditions. The aim is to establish a possible method for attenuating the current limitation of erythrocyte deformability to exercise in elite athletes in both normoxic and hypoxic conditions.

Hypotheses

1. Erythrocyte deformability will decrease from rest after hypoxic submaximal exercise
2. After chronic omega-3 PUFA supplementation (versus placebo), erythrocyte deformability will increase from pre-supplementation values
3. Microvascular oxygen delivery and oxygen extraction (as assessed by near-infrared spectroscopy) will increase in highly trained cyclists during submaximal exercise in both normoxia and hypoxia.

No changes in erythrocyte deformability or microvascular oxygenation are expected for those given the placebo treatment.

Delimitations

This study was delimited to the following:

1. Fifteen trained cyclists with a VO_2 max $\geq$ 55mL/kg/min.
2. The inspirate for experimental trials (normoxia or hypoxia) was randomized and blinded for each subject.
3. Subjects were blinded and randomized into placebo and supplementation groups
4. Subjects were required to make five visits to the lab: a preliminary graded maximal exercise(VO_2 max) test, two pre-tests in both normoxia and hypoxia, and two post-tests in both normoxia and hypoxia
5. Subjects performed each of the five trials at the same time of day.

Limitations

The results of this study were interpreted with the following limitations:

1. Subjects were selectively chosen to be highly trained.
2. The procedure followed subjects in the lab with a facemask attached during exercise, which is noticeably different than the usual training for athletes.
3. Trial took place with subjects while breathing inspirate with reduced oxygen levels (normobaric hypoxia), which is markedly different from competition/training these athletes may undergo at altitude (hypobaric hypoxia).
4. The findings from this study can only be attributed to a moderate altitude of approximately 3000m.

Assumptions

This study was based on the following assumptions:

1. Subjects accurately represented highly trained individuals.
2. Subjects did not change training status while undergoing testing.
3. Subjects followed treatment directions by ingesting the required daily dosage for the entire 6 weeks of supplementation.
4. Simulated altitude correlates with actual altitude.

Definitions

Erythrocyte Deformability: the physical characteristics of a red blood cell that permit it to change shape when under pressure, such as is required in the microcirculation.

Elongation Index: A measure of erythrocyte deformability.

Normoxia: Inspired air with an oxygen content of 0.2093

Hypoxia: Inspired air with an oxygen content less than 0.2093

Arterial hemoglobin oxygen saturation (SaO_2): The percentage of available hemoglobin binding oxygen.

CHAPTER 2: REVIEW OF LITERATURE

Oxygen Delivery to Skeletal Muscle during Exercise

The ability to perform endurance exercise is dependent on the transport of oxygen to the active skeletal muscles. It has been shown that the inability to increase oxygen delivery with increasing demand in locomotor muscles results in a limitation to aerobic capacity [14]. Bangsbo et al [15] investigated whether oxygen availability limits muscle oxygen uptake in the initial phase of intense exercise during a 3 minute bout of knee-extensor exercise. They found a delay in oxygen uptake after the onset of exercise, despite adequate oxygen delivery, as demonstrated in figure 1. The source of these limitations is suggested to be the misdistribution of blood flow in the exercising muscles limits, and not the transport of blood to the active tissues[15, 16].

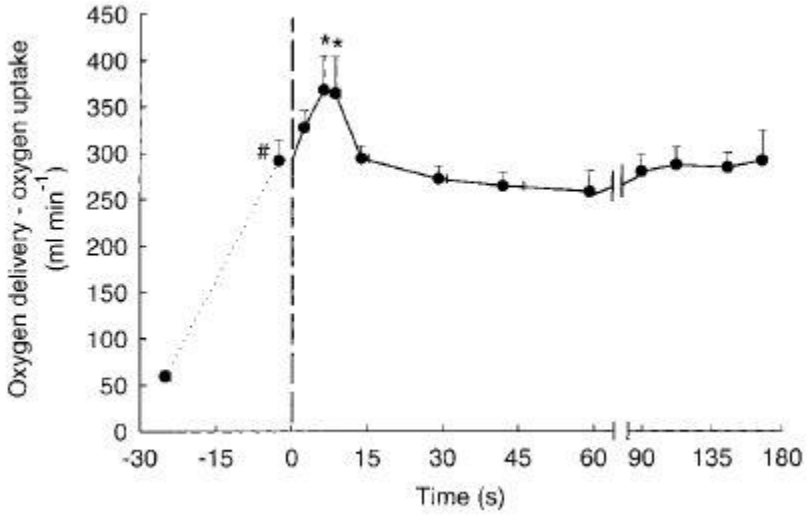

Figure 1. Difference between thigh oxygen delivery and thigh VO_2 during a three minute knee extensor exercise bout[15]

Upon the onset of skeletal muscle activity, blood flow increases rapidly. As the flow increases to the active tissue, additional capillary recruitment occurs, and blood perfuses into the microvasculature[17]. However, muscle capillary diameter is not greatly altered with increasing perfusion, therefore the oxygen-carrying erythrocytes must deform to allow for entry into the

microvasculature and the resulting appropriate distribution of increased blood flow to the tissue[18].

Erythrocyte Deformability

Erythrocyte deformability is the physical characteristics which permit a red blood cell, whose greater diameter normally exceed 7 μ, to travel through capillaries as narrow as 3 μ in diameter, and is thus an essential part in the delivery of oxygen to the tissues [1]. Within the narrow capillaries, the flexibility of erythrocytes is the most important determinant of blood flow to the tissue [2]. Alterations in these intrinsic properties of erythrocytes interfere with blood flow through the microcirculation [19]. Three factors have been discussed in contributing to the deformability of a red blood cell: 1) the biconcave shape, resulting in the high surface area-to-volume ratio of the cell, 2) the viscoelastic properties of the cell membrane, and 3) the viscosity of the hemoglobin solution within the cell [1].

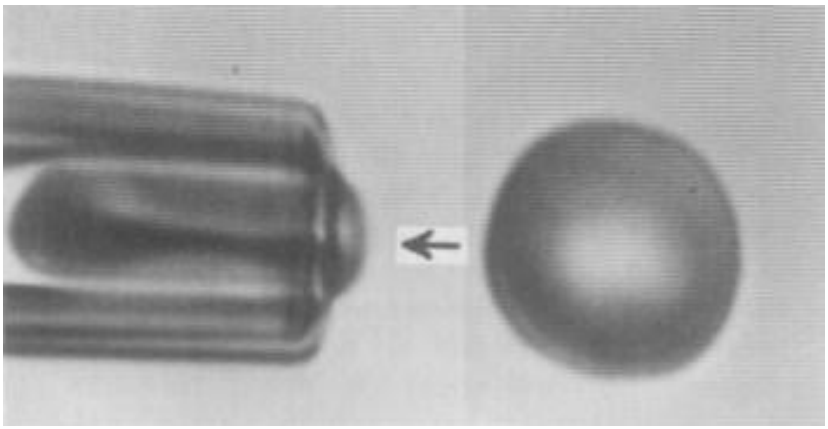

Figure 2. Video micrograph of an erythrocyte before and after entry into a small capillary [20].

The existing literature on erythrocyte deformability reports measurements from various techniques. One method commonly seen in the literature is erythrocyte filtration, which is defined by the ability of red blood cells to pass through a filter and is measured either by the time required for passage of a certain volume, or the volume of blood filtered in one minute[21]. The

filtration method passes a volume of blood through a membrane filter using a negative pressure, as seen in figure 3. The flow of blood through the apparatus is strongly influenced by the deformability of the erythrocytes as the diameter of the pores in the filter is less than that of a single red blood cell[21].

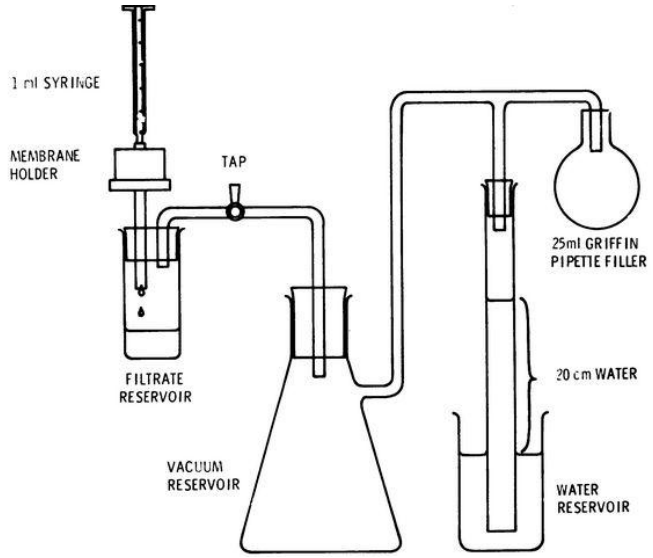

Figure 3. Apparatus for erythrocyte filtration used by Reid et al.[21]

Laser diffraction ellipsometry, or ektacytometry, is another frequently used method of measuring erythrocyte deformability. The erythrocytes are deformed to an ellipsoid shape and diffract a laser beam which passes through the test suspension and read by a detector, giving a measure of the elongation of the erythrocytes under a given shear stress[22], as demonstrated in figure 4. This method has demonstrated reproducibility, high precision, and narrow interassay variation[1].

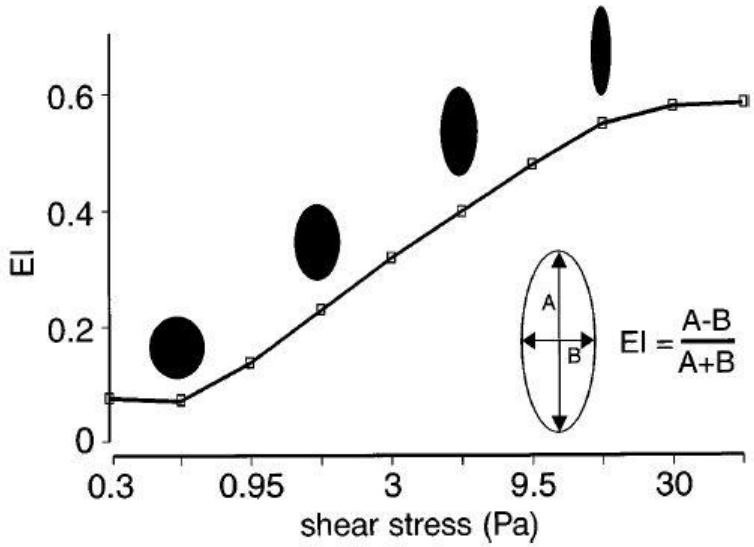

Figure 4. Changes in Elongation Index with increasing shear stress [23]

Research involving changes occurring in erythrocyte deformability has been conducted in many areas, including various diseases states[1, 7], the effect of drugs[1], varying levels of physical activity[8] and environmental conditions[3, 9-11]. For the purpose of this review, the focus was on how omega-3 Poly Unsaturated Fatty Acids (PUFA) supplementation, and hypoxia can elicit changes in erythrocyte deformability.

Omega-3 Polyunsaturated Fatty Acids

Following dietary supplementation with omega-3 Poly Unsaturated Fatty Acids (PUFA) containing docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) , an increase in erythrocyte deformability [12, 13, 24], reduction in RBC aggregation [25], and reduced whole blood viscosity [13] has been demonstrated. Due to the positive correlation between erythrocyte deformability and EPA content in erythrocytes elastic properties of RBC membrane dependent on proportion of EPA [12], and therefore it is suggested that the supplementation with omega-3s target the viscoelastic properties of the erythrocyte membrane to increase deformability.

The effects of PUFA supplementation on erythrocyte deformability and blood viscosity in healthy subjects was studied by Terano et al [12]. After four weeks of supplementation with a daily dosage of 3.6g EPA, they found a decrease in whole blood and red cell viscosity, and an increase in erythrocyte deformability. They also demonstrated a correlation between deformability and phospholipid content in the red cell membrane, as seen in figure 5. This finding supports the notion that the increase in deformability with PUFA supplementation is due to an increase in the viscoelastic properties of the cell membrane.

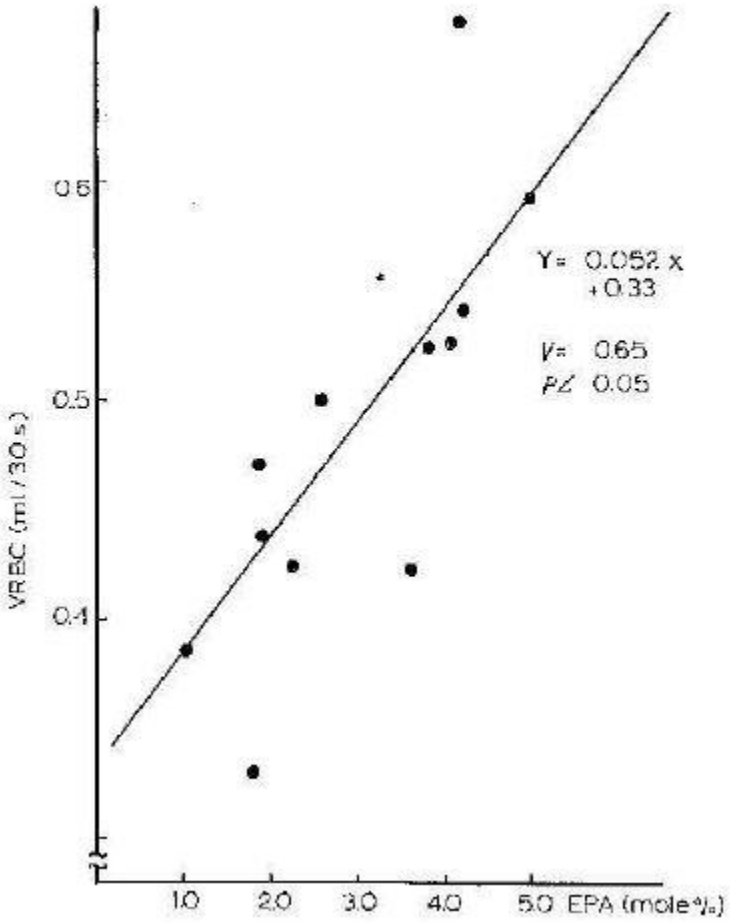

Figure 5. Correlation between erythrocyte deformability and EPA content in the red cell membrane.[12]

Cartwright et al [13] also investigated the effects of PUFA supplementation on erythrocyte deformability and blood viscosity in healthy subjects. A total of 3.4g of omega-3 were ingested each day, for a six week supplementation period. Test for erythrocyte phospholipid content, plasma and whole blood viscosity, and erythrocyte deformability were performed at 0, 3 and 6 weeks. An increase in filtration rate, indicating an increase in deformability, was seen at both three and six weeks, compared to baseline levels, as seen in figure 6. The analysis of erythrocyte phospholipids indicated an increase in membrane phospholipids after 6 weeks of supplementation with omega-3 PUFAs[13].

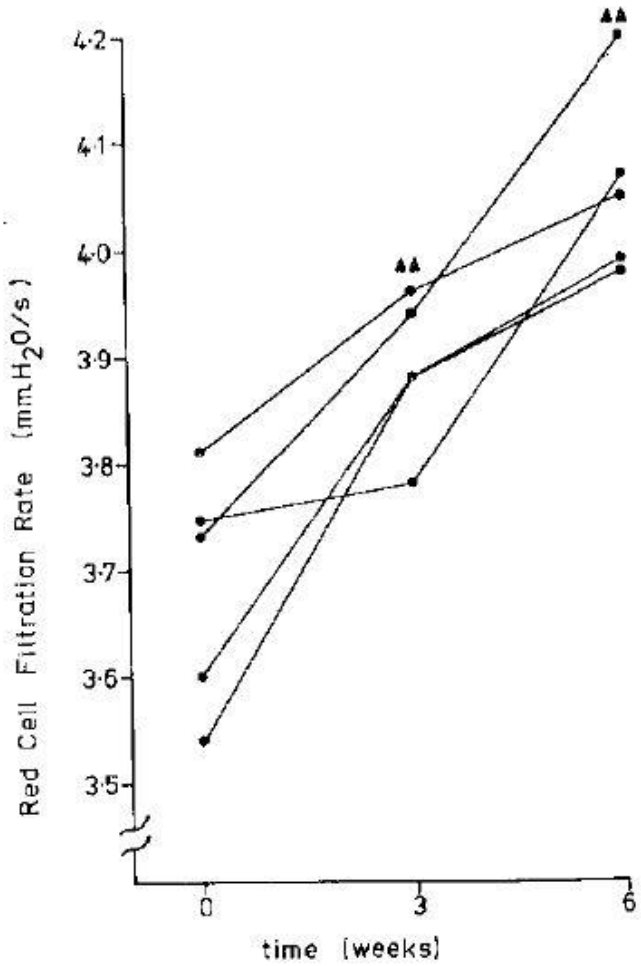

Figure 6. Changes in Red Cell Filtration Rate over the course of a six week PUFA supplementation

Due to the improvement in hematological factors, there have been attempts to demonstrate an increase in exercise performance with omega-3 supplementation [23]. It has been shown that erythrocyte deformability is higher in elite athletes than untrained controls [8], but the research on changes in deformability in highly trained individuals is currently limited. Oostenbrug et al. investigated the effects of PUFA supplementation on time trial performance in trained cyclists [12]. Although no improvement in performance was detected, no significant difference in erythrocyte deformability was demonstrated either, which is conflicting with previous findings. This could be due to a shortened supplementation time of only three weeks, where previous literature utilizes supplementation lengths of 4-12 weeks [12, 25], as well as a daily supplementation that totaled less than half the dosage of EPA used by Terano et al.

It has also been indicated that supplementation with PUFAs could result in an increase in VO_2 max [26]. A recent pilot study has found only one of 106 German elite winter athletes to be in the target range for the HS-Omega-3 Index , which is the percentage of the two omega-3 fatty acids (EPA) and (DHA) in total erythrocyte fatty acids [27], demonstrating the possibility of a strong impact on research involving the effects of PUFA supplementation on erythrocyte deformability in elite athletes by targeting membrane phospholipid content.

Hypoxia

Decreases in erythrocyte deformability produced by hypoxia have been demonstrated both in vitro [28] and in vivo [11]. Not only does erythrocyte deformability decrease with hypoxia, but these resulting changes in the red blood cells have been found to compromise the ability to perfuse the additional capillary recruitment that ordinarily results from hypoxia [3]. Through indicatory dilution techniques and transit time measures in the rat cremaster muscle, Parthasarathi and Lipowsky demonstrated that erythrocytes with decreased deformability

followed more central flow pathways, as opposed to perfusing the increased capillary recruitment within the microvasculature that was shown to occur with hypoxia [3].

To investigate how deformability is affected in hypoxia during exercise, Guezennec et al tested the effects of PUFA supplementation on physically trained subjects. The subjects performed 1 hour of exercise at 70% of VO_2 Max in both normoxic and hypoxic conditions. This test was performed both before and after 6 weeks of supplementation with 6g of PUFAs daily. As seen in figure 7, index of filtration increases in hypoxia and with submaximal exercise, but these differences are reduced with PUFA supplementation. It should be noted that index of filtration is a measure that increases as erythrocyte deformability decreases. These results demonstrated a decrease in deformability with exercise at hypoxia, and an attenuation of this decrease in deformability with PUFA supplementation [6].

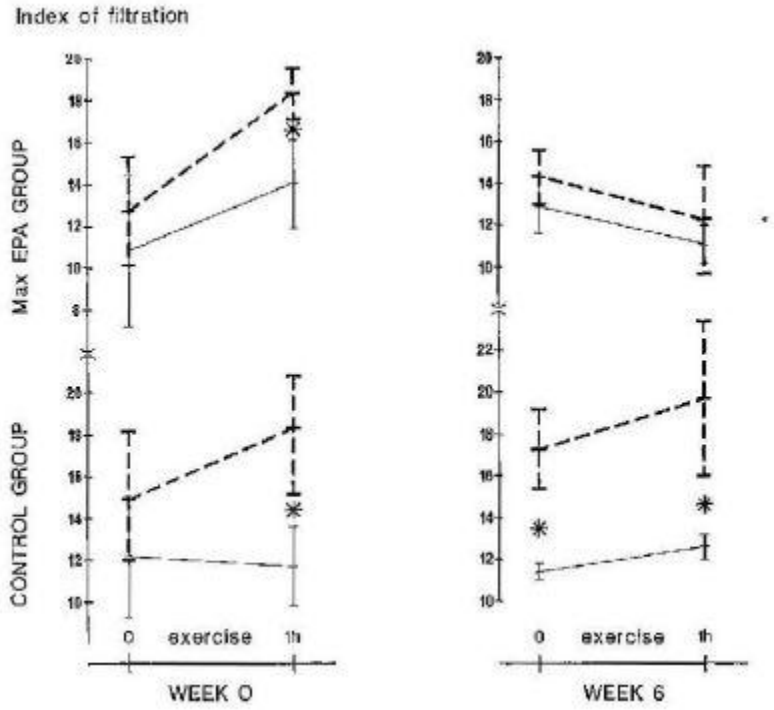

Figure 7. Changes in Filtration Index Pre- and Post- PUFA supplementation in normoxia (solid line) and hypoxia (dashed line)

Near Infrared Spectroscopy (NIRS)

NIRS is a method in which skeletal muscle oxygenation can be monitored noninvasively during exercise [29]. Near infrared spectroscopy (NIRS) is a technique which a near infrared light is emitted from source, which travels through tissue, then the wavelength of the light returning to the detector is measured, as demonstrated in figure 8.

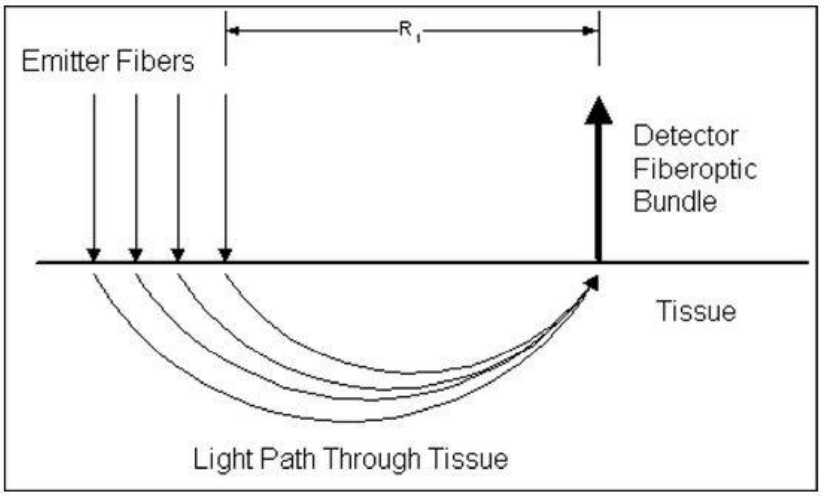

Figure 8. Schematic of method of action of NIRS[30]

NIRS takes advantage of the ability of near-infrared light to be absorbed by both the oxygenated and deoxygenated hemoglobin (Hb) in erythrocytes, and myoglobin (Mb) in skeletal muscle. The varying wavelengths at which these proteins absorb near infrared light allows NIRS to distinguish between the Oxy-Hb and Oxy-Mb, and the resulting measurements have been shown to be sensitive to dynamic trends in muscle oxygen delivery and intracellular O_2 availability in humans[31]. NIRS has been compared with measurements of femoral vein blood gases and appeared to show a similar pattern of muscle dexoygenation during exercise [32]. The technology has allowed for further investigation into the mechanisms regulating regional tissue blood flow [33], and has demonstrated consistent pattern in the rate of decrease in muscle oxygenation during incremental exercise [34]. By measuring hemoglobin levels in the active skeletal muscles, NIRS can be a useful tool in determining changes in oxygenation of the

microvasculature, and when used in conjunction ektacytometry, can be used to confirm whether changes in erythrocyte deformability result in improved oxygenation of the tissue.

CHAPTER 3: METHODOLOGY

The purpose of this study was to determine the effects of chronic omega-3 fatty acid supplementation on erythrocyte deformability and tissue oxygenation in highly trained cyclists during submaximal exercise in both normoxic and hypoxic conditions. The aim was to establish a possible method for attenuating the potential limitations of reduced erythrocyte deformability during exercise in trained endurance athletes in both normoxic and hypoxic conditions. The primary dependent variables of interest in this study are elongation index (EI), via Ektacytometry; and muscle oxygen saturation, oxy-hemoglobin + myoglobin (oxy-Hb+Mb), and deoxy-hemoglobin + myoglobin concentrations (deoxy-Hb+Mb), from near infrared spectroscopy (NIRS).

Subjects

The experiments were performed on highly trained cyclists (n=13). Sample size was determined from a power analysis using published data [6]. Highly trained status is defined as a VO_2 max ≥55ml/kg/min. Potential subjects who identify with any of the following were excluded from this study:

1. A history of smoking in the last twelve months
2. Omega-3 supplementation in the past 4 weeks, or fish consumption greater than one meal per week.
3. Women who are post-menopausal, taking birth control medication or hormone replacement therapy, or pregnant or possibly pregnant
4. A history of/current medical conditions such as pulmonary or cardiovascular disease.
5. Known allergy to omega-3 or omega-6 PUFAs

Experimental Design

Each subject will present for a total of five visits.

Figure 9. Overview of subject visits

During the initial visit subjects completed an informed consent form. Immediately following, a graded maximal exercise test on an electronically braked cycle ergometer (Velotron, RacerMate Inc., Seattle, WA) was performed to determine both VO_2 peak and peak power, to confirm whether the subject qualified as highly trained and establish the work rates for the subsequent visits, respectively. Subjects were informed of qualification at this point if the peak VO_2 taken from a 30 second average of the data exceeds 55ml/kg/min.

The subsequent two visits included three bouts submaximal cycling with the workloads of each bout set to 25, 50 and 75% of maximal power. The same protocol is to be followed for the second and third visits, with one performed in nomoxia, and the other in acute hypoxia (FIO_2 =15%; equivalent to 3000m / 9900ft altitude). During the hypoxic visit, the subject was exposed to the hypoxic gas starting 10 minutes prior to beginning exercise, and terminating upon completion of the final trial. Each of the submaximal cycling visits included the same exercise protocol, with each of the three bouts following this structure:

- Resting Data 1 minute
- Unloaded cycling 1 minute
- Loaded cycling 3 minutes
- Recovery Data 1 minute

During the final bout at 75% max power, after the initial 3 minutes of loaded cycling the subject continued until exhaustion, which was defined as an rpm below 60 or volitional exhaustion. A rest period of 5 minutes was given between each workload.

After the third visit, the subjects then underwent the 6-weeks supplementation period. The subjects were instructed to maintain their training status upon enrolment for the duration of the study. Following the six weeks of either PUFA or placebo supplementation, the subjects returned for two more visits, following the same protocol as visit two and three.

Treatment

Following enrolment, the subjects were pair matched by age and baseline EI, and were blinded and randomized into placebo and supplementation groups. The PUFA group took gel capsules totaling 2g of DHA, 3g of EPA, and 100mg of Vitamin E daily for 6 weeks, while the placebo group was given iso-caloric doses of safflower oil capsules, an omega-6 PUFA shown not to affect erythrocyte deformability or endothelial function [24, 35]. The subjects were instructed to take half the daily dose in the morning, and half the daily dose in the evening every day for six weeks. A journal was provided for the subjects to track their intake, to ensure compliance.

Measurements

Near Infrared Spectroscopy

NIRS (model 96208, ISS Inc., Champaign, IL) was used to determine oxygen saturation, oxy-hemoglobin+myoglobin, and deoxy-hemoglobin + myoglobin concentrations in the exercising muscle microcirculation by placing the detector on the vastus lateralis during the cycle protocol. A mark was made 15 cm proximal and5 cm lateral from the middle of the proximal border of the patella. The sensor was placed on that mark. The data of interest was

filtered, and an average was taken of the last 30 seconds each stage of all three workloads (rest, unloaded, loaded and recovery data).

Erythrocyte Deformability

Ektacytometry (RheoScan-D300, Sewon Meditech, Inc., Seoul, Korea) was used to measure the Elongation Index (EI) via finger prick capillary samples. A 5 µL sample of blood was taken for each measurement. The sample is placed under a given shear stress, and EI can be determined by measuring the laser diffraction pattern during shear, and is measured as

$$\frac{L - D}{L + D}$$

where L is the length and D the width of the RBC under deformation[36]. For each of the four experimental visits, finger prick samples were taken before beginning the exercise and after the completion of the cycling trials.

Metabolic Data

For all trials, subjects were fitted with a face mask (#2700, Hans Rudolph Inc., Shawnee, KS) attached to a two-way non-rebreathing valve. Breath by breath values for ventilation and expired gas concentrations were measured by a Vmax Encore Metabolic Cart (CareFusion Corporation, San Diego, CA) to sample the metabolic data. Arterial oxygen saturation (SaO_2) was monitored with fingertip pulse oximetry (Model PC-68A, Shenzen Creative Industry Co, Ltd., Shenzen, China). Heart rate was measured using a Polar heart rate monitor.

Data Analysis

A 2x2 split plot repeated measures ANOVA was done for each dependent variable of interest, with treatment (omega-3 or placebo) as the between groups factor, and time (pre- or post- supplementation) as the within groups factor. Tests of a priori sample main effects was also

done to determine differences in time within each treatment group, as well as differences in treatment groups for each time period. Statistical significance was set at an alpha of $p<0.05$.

CHAPTER 4: RESULTS

Subjects

A total of seventeen subjects were enrolled in the study. All of the subjects enrolled were able to surpass our inclusion criteria of a $\dot{V}O_2$ max of at least 55ml/kg/min, however, three subjects were unable to proceed past the initial $\dot{V}O_2$ max test, as we were unable to get an accurate reading from the NIRS. One subject had to be removed from the final analysis, as their training during supplementation was not comparable to the 6 weeks prior to the initial testing. From their training logs we observed a 25% increase in training volume over the course of the study. Subjects included for final analysis (n=13) had a $\dot{V}O_2$ max of 64.0 ± 4.9 ml/kg/min and reached a maximal power of 300.0 ± 54.2 W. Subject characteristics can be found in Table 1.

Table 1 – Subject Characteristic Data

	Omega-3 PUFA (n=6)	**Placebo (n=7)**	**Grouped (n=13)**
Age	20±1.2	21.1±2.2	20.7±1.9
Height	177.6±7.0	181.8±3.7	179.8±5.7
Mass	78.9±6.7	77.6±5.9	78.21±6.3
VO_2max	60.2±4.1	67.3±2.6	64.0±4.9
Max Power	379±64	389±44	385±54

Table 1. Subject characteristics measures taken upon initial visit.

Erythrocyte deformability

Data for erythrocyte deformability in the pre-supplementation hypoxic condition can be found in Table 2. When comparing baseline values to measurements taken post-exercise in the hypoxic condition prior to supplementation, there were no significant differences in Elongation Index (EI). Data for baseline erythrocyte deformability can be found in Table 3. After supplementation, there were no significant differences in baseline EI when compared to pre-supplementation values.

Table 2 – Hypoxic Erythrocyte Deformability Data

	Omega-3 PUFA (n=6)		**Placebo (n=7)**		**Grouped (n=13)**	
	Baseline	***Post-Exercise***	***Baseline***	***Post-Exercise***	***Baseline***	***Post-Exercise***
Shear Stress						
5 Pa	0.320± 0.006	0.320±0.006	0.318±0.009	0.320±0.008	0.319±0.008	0.320±0.007
10 Pa	0.384±0.009	0.382±0.006	0.383±0.014	0.387±0.012	0.383±0.012	0.385±0.010
15 Pa	0.472±0.008	0.472±0.006	0.473±0.011	0.477±0.011	0.473±0.009	0.475±0.009
20 Pa	0.571±0.006	0.574±0.004	0.576±0.011	0.576±0.004	0.574±0.009	0.575±0.004

Erythrocyte deformability measures as measured via Ektacytometry (Elongation Index) at 5, 10, 15, and 20 Pa sheer stress. Baseline, baseline (resting) measures prior to 6-week supplementation period; Post-Exercise, measures following exercise protocol in Hypoxia (FiO2 = 15.0%), prior to 6-week supplementation period.
Table 2. Pre-Supplementation deformability measures in hypoxic condition.

Table 3 – Baseline Erythrocyte Deformability Data

	Omega-3 PUFA (n=6)		Placebo (n=7)		Grouped (n=13)	
	Pre	***Post***	***Pre***	***Post***	***Pre***	***Post***
Shear Stress						
Normoxic Trial						
5 Pa	0.321±0.008	0.307±0.032	0.325±0.006	0.321±0.008	0.323±0.007	0.315±0.023
10 Pa	0.386±0.010	0.380±0.018	0.390±0.009	0.386±0.011	0.388±0.010	0.383±0.015
15 Pa	0.476±0.009	0.470±0.017	0.480±0.007	0.476±0.011	0.478±0.008	0.473±0.014
20 Pa	0.575±0.002	0.564±0.190	0.577±0.005	0.575±0.009	0.576±0.004	0.570±0.016
Hypoxic Trial						
5 Pa	0.320±0.006	0.322±0.004	0.318±0.009	0.321±0.006	0.319±0.008	0.321±0.006
10 Pa	0.384±0.009	0.385±0.007	0.383±0.014	0.385±0.007	0.383±0.012	0.385±0.007
15 Pa	0.472±0.008	0.475±0.006	0.473±0.011	0.476±0.006	0.473±0.009	0.476±0.006
20 Pa	0.571±0.006	0.577±0.005	0.575±0.011	0.577±0.008	0.574±0.009	0.577±0.007

Baseline erythrocyte deformability measures as measured via Ektacytometry (Elongation Index) at 5, 10, 15, and 20 Pa sheer stress. Pre, baseline (resting) measures prior to 6-week supplementation; Post, baseline measures following six weeks of supplementation of omega-3 PUFA, or safflower oil (placebo); PUFA, polyunsaturated fatty-acids.
Table 3. Baseline deformability measures pre- and post-supplementation.

Data for post- exercise erythrocyte deformability can be found in Table 4. We demonstrated a significant difference in elongation index (EI) measures taken at 20 Pascals in the hypoxic condition between Omega-3(0.580±0.002) and Placebo(0.574±0.006) groups in post-exercise measures following the 6-week supplementation. We also identified a significant increase in EI measures post-supplementation (0.580±0.002) compared to pre-supplementation (0.574±0.002) with the treatment group after exercise for the hypoxic condition. There was no significant difference between the pre- and post-supplementation values for the control group. We did not see a change in deformability at lower sheer stresses in the hypoxic condition, nor did we see any changes in in the normoxic condition.

Table 4 – Post- Exercise Erythrocyte Deformability Data

	Omega-3 PUFA (n=6)		Placebo (n=7)		Grouped (n=13)	
	Pre	***Post***	***Pre***	***Post***	***Pre***	***Post***
Shear Stress						
Normoxic Trial						
5 Pa	0.321±0.007	0.321±0.006	0.322±0.008	0.322±0.003	0.321±0.007	0.322±0.005
10 Pa	0.386±0.010	0.386±0.008	0.389±0.110	0.385±0.005	0.388±0.011	0.385±0.006
15 Pa	0.476±0.009	0.475±0.009	0.478±0.009	0.476±0.005	0.477±0.009	0.475±0.007
20 Pa	0.576±0.003	0.572±0.008	0.574±0.004	0.578±0.003	0.575±0.003	0.575±0.007
Hypoxic Trial						
5 Pa	0.320±0.006	0.323±0.006	0.320±0.008	0.320±0.009	0.320±0.007	0.322±0.008
10 Pa	0.382±0.006	0.388±0.006	0.387±0.012	0.385±0.009	0.385±0.010	0.386±0.008
15 Pa	0.472±0.006	0.480±0.005	0.477±0.011	0.475±0.007	0.475±0.009	0.477±0.007
20 Pa	0.574±0.004	*# 0.580±0.002	0.546±0.004	0.574±0.006	0.575±0.004	0.577±0.006

Post- exercise erythrocyte deformability measures as measured via Ektacytometry (Elongation Index) at 5, 10, 15, and 20 Pa sheer stress. Pre, baseline (resting) measures prior to 6-week supplementation; Post, baseline measures following six weeks of supplementation of omega-3 PUFA, or safflower oil (placebo); PUFA, polyunsaturated fatty-acids.

* significantly different from pre-supplementation ($p > 0.05$), #significantly different from placebo ($p > 0.05$),

Table 4. Post-exercise deformability measures pre- and post-supplementation.

NIRS

In order to ensure we had an appropriate comparison given different baseline levels between subjects, for the NIRS data analysis we used the difference between the unloaded cycling prior to the first stage (25% of maximal power) and the end of exercise for each of the three stages, respectively. Figure 10 shows the NIRS data tracing during a typical exercise trial. The data collected during the normoxic trials can be found in table 5.

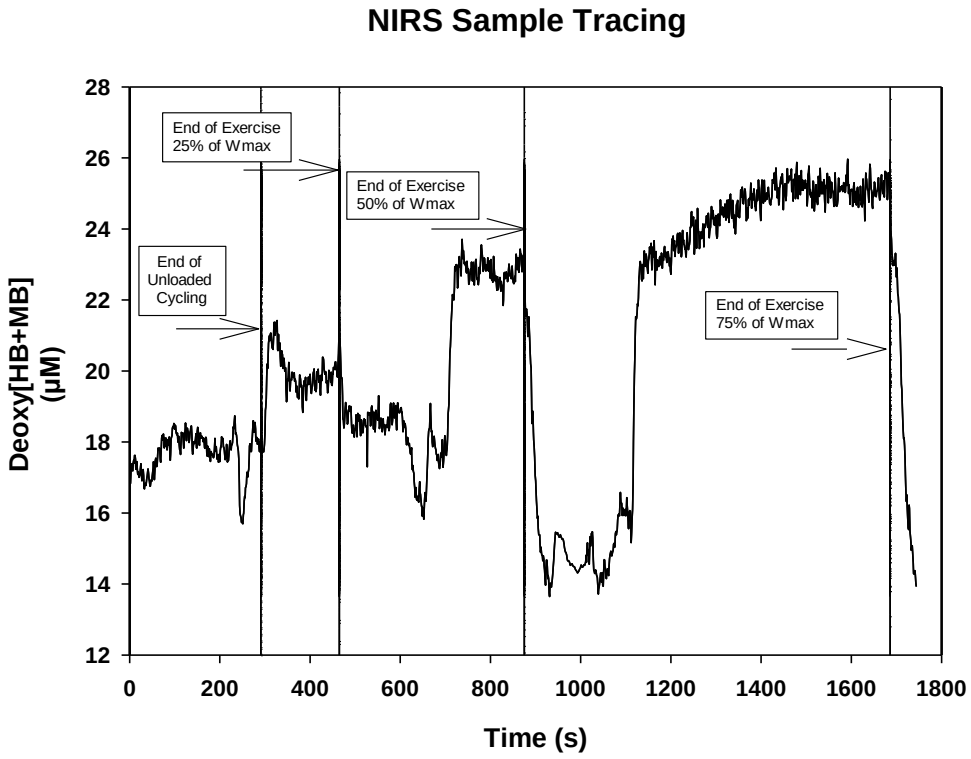

Figure 10. A sample tracing of the NIRS data collected during each of the exercise trials.

Table 5 – Near-Infrared Spectroscopy Data from Normoxic (F_IO_2 = 20.93%) Exercise Trials

	Omega-3 PUFA (n=6)		Placebo (n=7)		Grouped (n=13)	
	Pre	***Post***	***Pre***	***Post***	***Pre***	***Post***
Δ[OxyHb +Mb] (μM)						
25% of Peak Power	0.88±1.56	1.38±1.67	0.07±1.34	0.53±5.21	0.45±1.50	0.92±4.01
50% of Peak Power	-1.72 ±2.71	-2.53±1.90	-3.37±3.61	-0.53±5.18	-2.61±3.31	-1.51±4.13
75% of Peak Power	-1.58±3.14	-3.65±1.98	-7.07±6.26	-2.94±5.25	-4.54±5.76	-3.27±4.09
Exhaustion	-2.80±3.82	-4.22±5.40	-8.24±6.96	-5.83±5.37	-5.73±6.34	-5.08±5.44
Δ [DeoxyHb +Mb] (μM)						
25% of Peak Power	1.72±0.74	-0.57±7.60	3.60±1.17	4.66±3.14	2.73±1.37	2.25±6.23
50% of Peak Power	4.85±2.02	5.45±3.64	9.53±3.21	9.30±4.57	7.37±3.59	7.52±4.59
75% of Peak Power	7.75±3.82	9.32±5.16	14.50±6.69	12.86±6.78	11.38±6.49	11.22±6.34
Exhaustion	12.95±5.56	14.72±7.11	19.20±7.36	19.26±11.17	16.32±7.29	17.16±9.78
Δ [Total Hb +Mb] (μM)						
25% of Peak Power	2.67±1.99	4.25±1.71	3.66±1.53	5.19±3.26	3.20±1.83	4.75±2.70
50% of Peak Power	3.17±4.16	4.53±3.05	5.84±3.57	8.66±5.91	4.61±4.08	6.75±5.23
75% of Peak Power	6.20±5.58	5.62±3.79	7.41±3.07	9.91±8.16	6.85±4.45	7.93±6.86
Exhaustion	10.82±9.85	10.40±6.49	10.93±3.82	13.44±11.04	10.88±7.26	12.04±9.35
Δ Tissue Saturation (%)						
25% of Peak Power	-1.32±0.53	-2.55±2.06	-2.90±1.12	-3.43±3.63	-2.17±1.20	-3.02±3.04
50% of Peak Power	-5.60±0.83	-8.10±1.76	-8.51±2.65	-6.87±3.53	-7.17±2.49	-7.44±2.92
75% of Peak Power	-8.13±2.13	-9.88±2.68	-13.67±7.45	-10.03±3.95	-11.11±6.90	-9.96±3.42
Exhaustion	-13.95±1.04	-14.43±3.94	-17.03±7.83	-14.61±5.07	-15.61±5.99	-14.53±4.58

The change in NIRS measures for each workload when compared to unloaded exercise prior to the first workload
Pre, baseline testing prior to 6-week supplementation; Post, testing following six weeks of supplementation of omega-3 PUFA, or safflower oil (placebo); PUFA, polyunsaturated fatty-acids.
Table 5. Near-Infrared Spectroscopy (NIRS) measures pre- and post-supplementation in Normoxic condition.

Pre-Supplementation versus Post-Supplementation values in Normoxic Condition

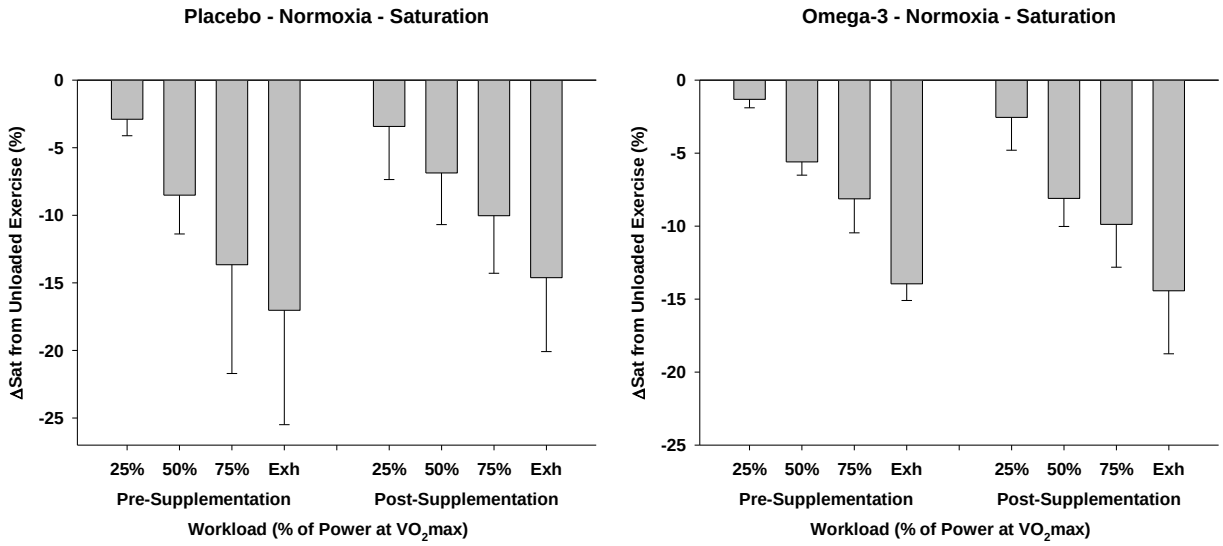

Figure 11. Change in tissue saturation measures at four exercise intensities in Normoxia pre- and post-supplementation

Figure 11 displays the changes in Tissue Saturation (%) from unloaded exercise to 25%, 50%, 75% of max power and at exhaustion (Max) in Normoxia in the Placebo group (left) and Omega-3 group (right). All comparisons for tissue saturation measures showed no significant differences within groups.

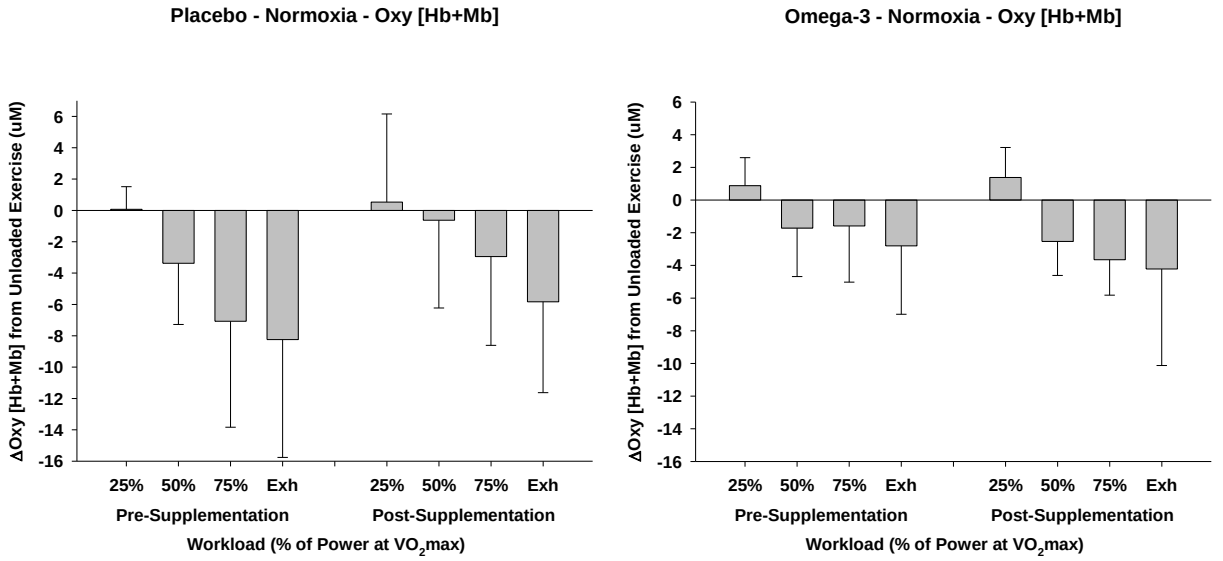

Figure 12. Change in Oxy-Hemoglobin measures at four exercise intensities in Normoxia pre- and post-supplementation

Figure 12 displays the changes in Oxy [Hb+Mb] from unloaded exercise to 25%, 50%, 75% of max power and at exhaustion (Max) in Normoxia in the Placebo group (left) and Omega-3 group (right). There were no significant differences within groups between Pre- and Post-Supplementation values at any common workload.

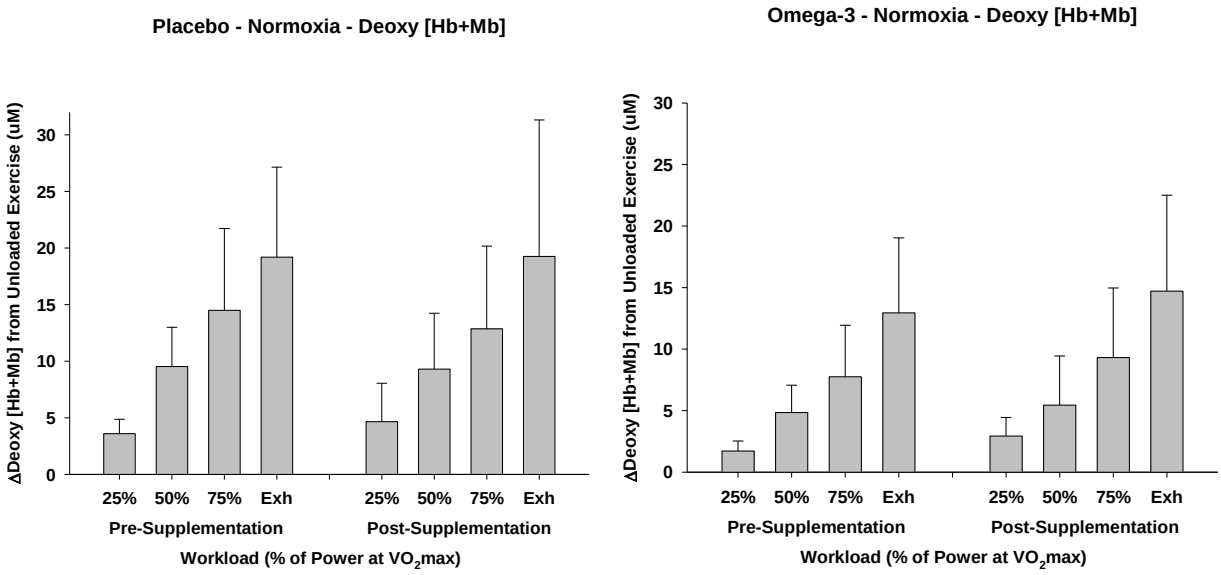

Figure 13. Change in Deoxy-Hemoglobin measures at four exercise intensities in Normoxia pre- and post-supplementation

Figure 13 displays the changes in Deoxy [Hb+Mb] from unloaded exercise to 25%, 50%, 75% of max power and at exhaustion (Max) in Normoxia in the Placebo group (left) and Omega-3 group (right). There were no significant differences between Pre- and Post-Supplementation values at any common workload within groups.

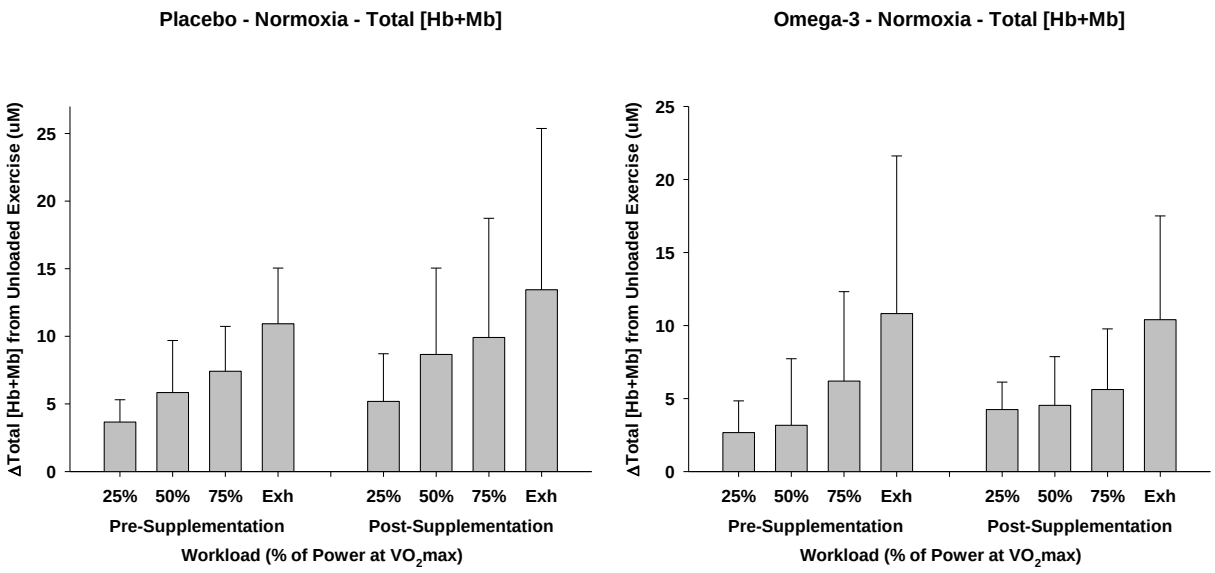

Figure 14. Change in total hemoglobin measures at four exercise intensities in normoxia pre- and post-supplementation

Figure 14 displays the changes in Total [Hb+Mb] from unloaded exercise to 25%, 50%, 75% of max power and at exhaustion (Max) in Normoxia in the Placebo group (left) and Omega-3 group (right). There were no significant differences between Pre- and Post-Supplementation values at any common workload within groups.

Pre-Supplementation versus Post-Supplementation values in Hypoxia

The data collected during the hypoxic trials can be found in table 6. Figure 15 displays the changes in tissue saturation (%) from Pre- to Post-Supplementation in Placebo (left) and Omega-3 (right) groups at each workload. At each common workload, there were no significant differences between Pre- and Post-Supplementation values within groups.

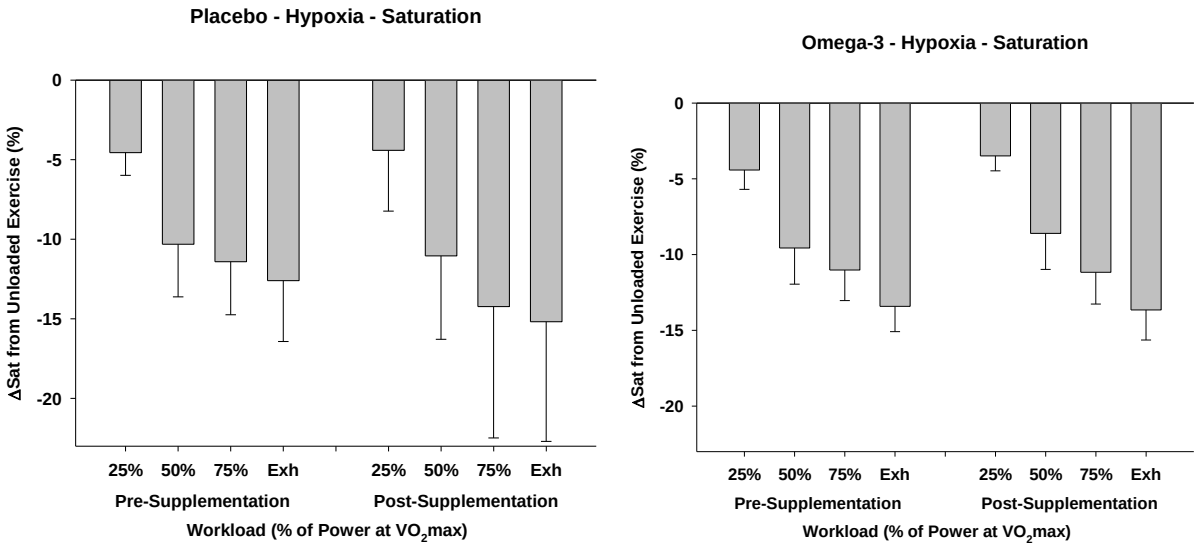

Figure 15. Change in tissue saturation measures at four exercise intensities in hypoxia pre- and post-supplementation

Table 6 – Near-Infrared Spectroscopy Data from Hypoxic (F_IO_2 = 15.0%) Exercise Trials

	Omega-3 PUFA (n=6)		Placebo (n=7)		Grouped (n=13)	
	Pre	***Post***	***Pre***	***Post***	***Pre***	***Post***
Δ[OxyHb +Mb] (μM)						
25% of Peak Power	-2.03±1.30	-0.17±1.51	-1.03±2.10	-1.67±2.76	-1.49±1.84	-0.98±2.40
50% of Peak Power	-4.55±2.53	-3.78±3.14	-5.79±2.75	-6.29±4.32	-5.22±2.72	-5.13±4.02
75% of Peak Power	-4.52±3.28	-4.93±2.84	-7.13±4.96	-8.88±5.07	-5.92±4.47	-7.06±4.63
Exhaustion	-5.75 ±3.43	-5.03±2.97	-7.86 ±6.09	-9.04±6.17	-6.88±5.15	-7.19±5.35
Δ [DeoxyHb +Mb] (μM)						
25% of Peak Power	3.35±2.14	3.35±1.29	5.11±1.45	5.60±4.60	4.53±1.91	4.56±3.66
50% of Peak Power	8.28±3.84	7.38±3.50	10.77±4.46	12.45±7.23	9.62±4.37	10.12±6.34
75% of Peak Power	9.92±4.31	9.90±4.81	11.41±4.47	15.20±10.11	10.72±4.46	12.75±8.53
Exhaustion	12.10±5.01	13.00±6.21	12.81±4.58	18.07±11.20	12.48±4.80	15.73±9.58
Δ [Total Hb +Mb] (μM)						
25% of Peak Power	1.82±2.12	3.20±1.98	4.04±2.05	3.91±2.58	3.01±2.36	3.58±2.35
50% of Peak Power	3.75±3.33	3.58±3.47	5.00±3.16	6.16±3.83	4.42±3.30	4.97±3.89
75% of Peak Power	5.42±5.30	5.00±4.83	4.27±5.22	6.31±7.71	4.80±5.29	5.71±6.57
Exhaustion	6.37±5.98	7.93±7.23	4.96±4.68	9.03±8.71	5.61±5.37	8.52±8.08
Δ Tissue Saturation (%)						
25% of Peak Power	-4.42±1.17	-3.48±0.90	-4.56±1.32	-4.41±3.53	-4.49±1.25	-3.98±2.70
50% of Peak Power	-9.57±2.17	-8.60±2.17	-10.31±3.06	-11.04±4.86	-9.97±2.71	-9.92±4.04
75% of Peak Power	-11.02±1.84	-11.17±1.91	-11.41±3.08	-14.23±7.64	-11.23±2.59	-12.82±5.96
Exhaustion	-13.40±1.52	-13.65±1.81	-12.60±3.54	-15.19±6.96	-12.98±2.83	-14.48±5.31

The change in NIRS measures for each workload when compared to unloaded exercise prior to the first workload
Pre, baseline testing prior to 6-week supplementation; Post, testing following six weeks of supplementation of omega-3 PUFA, or safflower oil (placebo); PUFA, polyunsaturated fatty-acids.
Table 6. Near-Infrared Spectroscopy (NIRS) measures pre- and post-supplementation in hypoxic condition.

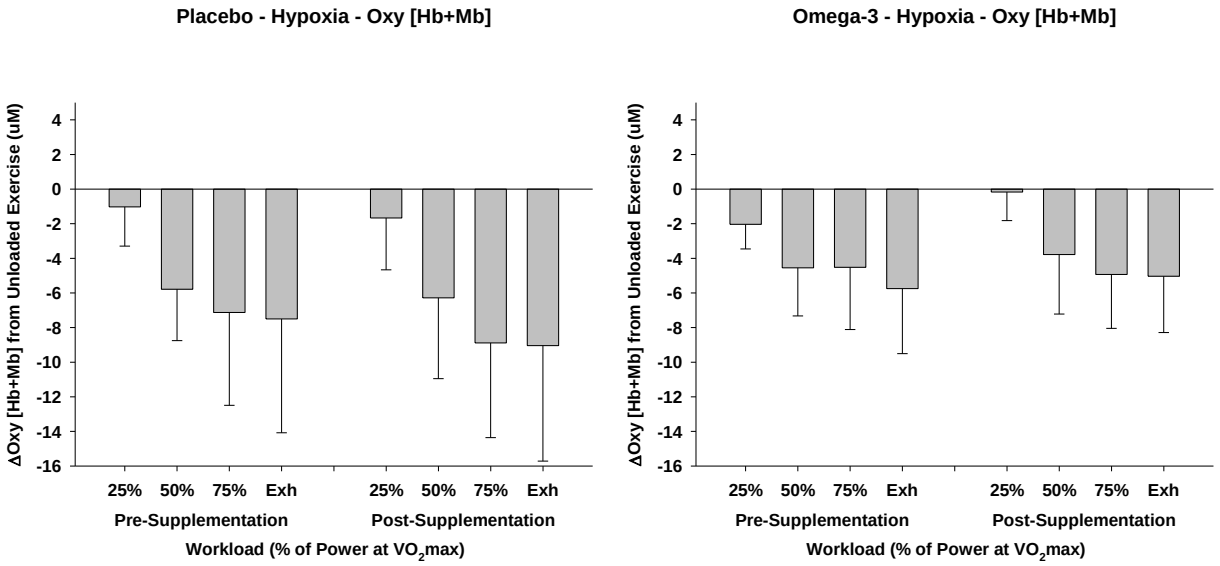

Figure 16. Change in oxy-hemoglobin measures at four exercise intensities in hypoxia pre- and post-supplementation.

Figure 16 displays the changes in Oxy [Hb+Mb] from Pre- to Post-Supplementation in Placebo (left) and Omega-3 (right) groups at each workload. At each common workload, there were no significant differences between Pre- and Post-Supplementation values within groups.

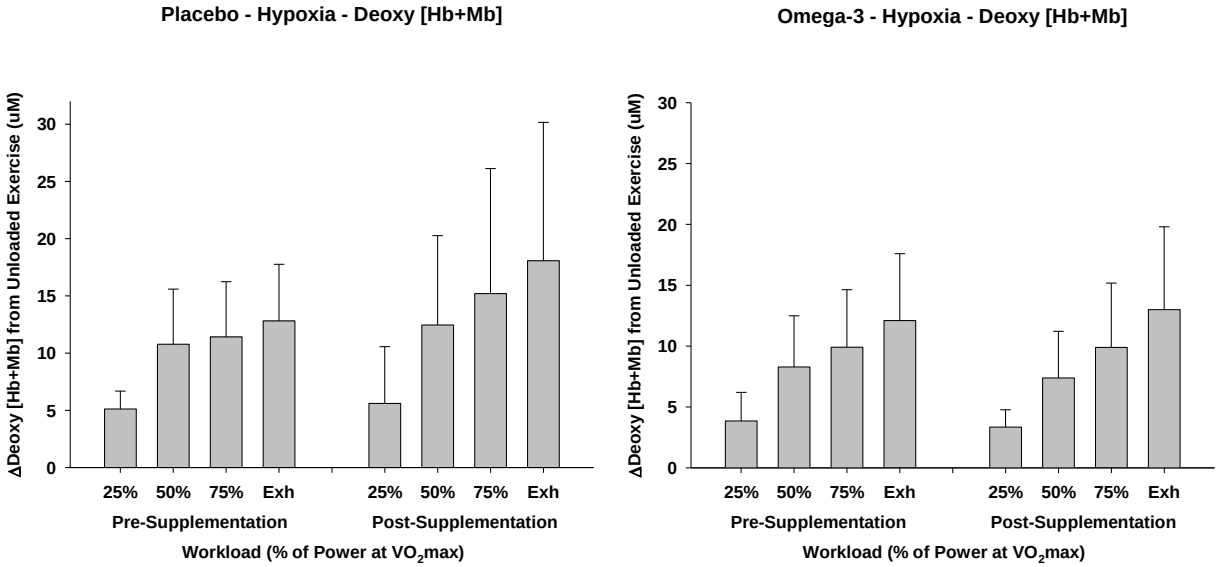

Figure 17. Change in deoxy-hemoglobin measures at four exercise intensities in hypoxia pre- and post-supplementation

Figure 17 displays the changes in Deoxy [Hb+Mb] from Pre- to Post-Supplementation in Placebo (left) and Omega-3 (right) groups at each workload. At each common workload, there were no significant differences between Pre- and Post-Supplementation values within groups.

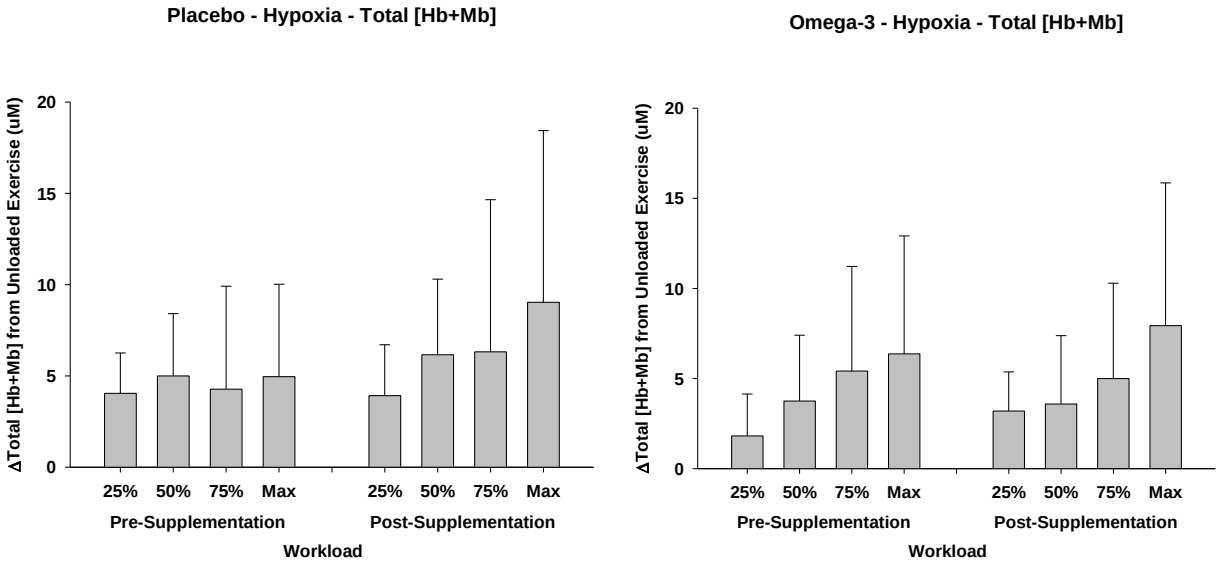

Figure 18. Change in total hemoglobin measures at four exercise intensities in hypoxia pre- and post-supplementation

Figure 18 displays the changes in Total [Hb+Mb] from Pre- to Post-Supplementation in Placebo (left) and Omega-3 (right) groups at each workload. At each common workload, there were no significant differences between Pre- and Post-Supplementation values within groups.

CHAPTER 5: DISCUSSION

The aim of this study was to determine the effects of chronic omega-3 poly-unsaturated fatty acid (PUFA) supplementation on erythrocyte deformability and tissue oxygenation in highly trained cyclists during submaximal exercise in both normoxic and hypoxic conditions. During exercise, as regional blood flow increases, blood perfuses into the microvasculature in order to supply meet the increased oxygen demand of the working muscles. The smallest of the capillaries can be as narrow as 3 μm in diameter [1] and since erythrocytes are, on average, 7μm in diameter, the tissues supplied by the microvasculature rely on the ability of erythrocytes to deform for the delivery of oxygen [2]. In cases where the erythrocytes are unable to adequately deform, they bypass the most narrow vessels of the microvasculature [3], thus causing areas of skeletal muscle to lack adequate oxygenation. In order for maximal oxygen delivery to occur in the active skeletal muscle, erythrocyte deformability must be maintained to allow for passage through the microvasculature. Our data indicate that there is neither a change in baseline erythrocyte deformability nor an attenuation of deformability decrease with exercise in hypoxia following PUFA supplementation, as indicated by Elongation Index measures. The data also failed to demonstrate any changes in microvascular tissue oxygenation via NIRS. This would suggest that omega-3 PUFA supplementation is likely not an advantage to mitigate endurance exercise performance impairment with acute exposure to altitude.

A correlation has been demonstrated between erythrocyte deformability and phospholipid content in the cell membrane [12], and it has been demonstrated that chronic supplementation of PUFAs increases erythrocyte deformability and skeletal muscle blood flow [13]. However, whether any omega-3 mediated increase in erythrocyte deformability leads to improvements in microvascular oxygenation during exercise and/or hypoxic exposure has not been previously demonstrated. By using ektacytometry to quantify erythrocyte deformability and NIRS to

evaluate tissue oxygenation during exercise, we intended to add novel information to the existing literature on omega-3 fatty acid supplementation and erythrocyte deformability.

Erythrocyte Deformability

Omega-3 supplementation has been shown to increase erythrocyte deformability by targeting the viscoelastic properties of the erythrocyte membrane. This is due to the incorporation of the polyunsaturated fatty acids into the cell membrane, which contributes to a more fluid cell membrane, allowing the cell to be more deformable [12]. Our findings did not support the hypothesis that deformability would decrease with exercise in hypoxia and increase with PUFA supplementation, which conflicts with previous findings [13]. There are a few primary differences between our protocol and those who were able to demonstrate a change in deformability: measurement method, subject fitness levels, and exercise protocol.

Despite having similar supplementation length and daily dosage of EPA, previous studies were able to demonstrate a significant difference in erythrocyte deformability following PUFA supplementation [13]. However, these studies employed a different measurement method to quantify erythrocyte deformability. First described by Reid et al, a filtration method [21] was commonly used to observe changes erythrocyte deformability prior to Ektacytometry becoming more prevalent in the literature. Where ektacytometry measures elongation index, the filtration method provides red cell filtration rate, or index of filtration, as an indicator of deformability. In using ektacytometry, we were unable to demonstrate a change in deformability with PUFA supplementation. However, recent research into deformability measurement methods has demonstrated not only do the two different measurement techniques not correlate with each other, and they suggest that erythrocyte deformability is a "property that is operationally defined by the measurement methodology" [37], therefore despite using similar study designs, we still may not expect similar results due to varying measurement techniques.

For the studies showing positive changes in deformability with PUFA supplementation [13], subjects were defined as healthy male subjects, whereas our subjects were highly trained cyclists ($\dot{V}O_2$max= 64.0 ±4.9 mL/kg/min). It has been suggested that erythrocyte deformability is higher in elite athletes than untrained controls [8]. Further research has attempted to demonstrate differences in erythrocyte deformability with varying types of sport[38], but the subjects did not perform a $\dot{V}O_2$max test, so it remains unclear whether there is a relationship between erythrocyte deformability and endurance levels. There is the possibility that due to their fitness levels, our subjects had already reached their maximal deformability levels, and were therefore not aided by the PUFA supplementation.

One previous study with similarly trained subjects ($\dot{V}O_2$max= 58 ±6 mL/kg/min) was able to demonstrate a decrease in deformability with exercise at hypoxia, and an attenuation of this decrease in deformability with PUFA supplementation [6]. However, this study differed in methodology in a number of ways. They measured deformability via the filtration method and while using simulations of similar altitudes (3000m), they utilized a hypobaric chamber, where our methodology utilized normobaric hypoxia. The exercise protocol also varied, as they had their subjects cycle at an intensity equivalent to 70% of their $\dot{V}O_2$max for 1 hour, so it is possible that the prolonged exercise elicited a stronger response, which was able to demonstrate significant differences between supplementation and placebo groups.

To our knowledge, there is only one study that has previously researched the changes in erythrocyte deformability with omega-3 supplementation in highly trained subjects with ektacytometry as their measurement method, however they were unable to demonstrate changes in erythrocyte deformability with PUFA supplementation in "well- trained" cyclists ($\dot{V}O_2$max= 4.4 ± 0.1 L/min) [23]. Despite using the sample measurement technique, they varied in

supplementation composition and length, and utilized a prefixed absolute workload test set at 70% of the subjects Wmax for their exercise protocol.

Due to the variety of research protocol within the present body of literature, it is difficult to make comparisons between findings. From differing measurement techniques [6, 12, 13], subject fitness levels [12, 13], and exercise protocol [6, 23], it is evident that further research needs to be completed to determine the relationship between erythrocyte deformability and omega-3 supplementation in highly trained subjects.

NIRS

By measuring hemoglobin levels in the active skeletal muscles, NIRS can be a useful tool in determining changes in oxygenation of the microvasculature, and when used in conjunction ektacytometry, can be used to confirm whether changes in erythrocyte deformability result in improved oxygenation of the tissue. The research exploring the effect of erythrocyte deformability on microvascular oxygen delivery in highly trained individuals has not been extensively pursued and whether any omega-3 mediated increase in erythrocyte deformability leads to improvements in microvascular oxygenation during exercise and/or hypoxic exposure is currently unknown.

Since we were unable to demonstrate a decrease in deformability with exercise in hypoxia, or a treatment effect of the omega-3 supplementation on baseline erythrocyte deformability, and only a marginal significant difference on post-exercise erythrocyte deformability measures, we would not expect to be able to demonstrate significant differences in microvascular tissue oxygenation.

Limitations

Since there are many factors that influence erythrocyte deformability that can vary between individuals, an ideal methodology for this study would include a crossover design, with a washout period between supplementations and retest after the second supplementation period. Logistical concerns of subject recruitment and adherence over the longer time period were the cause of not selecting this design. The power analysis done prior to testing indicated 15 subjects would be necessary and the final subject number of this study was two short at 13, however based on the data collected it is unlikely that additional subjects would have altered the results.

Since our subjects were all highly trained athletes, our results depending on their training remaining consistent between the six weeks prior to supplementation and the six week supplementation period. As demonstrated by the subject that was removed from the final analysis, finding a twelve week period where there are no changes in training levels for highly trained athletes can difficult to achieve.

In order to keep record of the subjects' supplementation adherence, they completed a supplementation log and were instructed to return the pills remaining at the end of the study. The levels of polyunsaturated fatty acids in their blood were not analyzed before and after supplementation, so their compliance cannot be confirmed, which could have altered our results.

In comparing findings to previous research that has shown changes in erythrocyte deformability in highly trained individuals with PUFA supplementation, the lack of significance could be due to varying hypoxic exposure. A simulated altitude of 3000m in hypobaric hypoxia was used in previous research [6], and while we maintained a simulated altitude of 3000m, we did so with normobaric hypoxia with FIO_2 =15%. Being that there is a potential for difference in results between normobaric and hypobaric hypoxia, a higher simulated altitude may have been necessary to see similar results.

Future Directions

There are several novel aspects to this investigation, and therefore are many areas that merit further investigation. To our knowledge, we are the first to examine the changes of erythrocyte deformability with polyunsaturated fatty acid supplementation in highly trained individuals utilizing ektacytometry as a measurement methodology, alongside with confirming the effects on microvascular tissue oxygenation with near infrared spectroscopy (NIRS). Future research with these methodologies could add to the field of knowledge surrounding erythrocyte deformability and performance.

Since there is also limited knowledge on erythrocyte deformability in highly trained individuals and further research is necessary to confirm the deformability levels of highly trained athletes and determine whether there is room for improvement with omega-3 supplementation. Comparison between subjects with varied fitness levels could help bring insight into the effect of training status on erythrocyte deformability. This may result in finding that elite athletes may not benefit, but there may be room for a performance aid for recreational athletes as well a potential clinical application for exercise tolerance in untrained individuals.

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SUPPLEMENTAL MATERIALS

Appendix A: Subject Characteristic Data

Subject	Supplement	Age	Sex	Height (cm)	Mass (kg)	VO_2max (mL/kg/min)	MaxPower (W)
1015	Omega-3	20	M	183.0	82.1	68.6	450
1017	Omega-3	18	M	175.5	72.5	61.7	350
1020	Omega-3	20	F	168.7	68.0	56.8	300
1021	Placebo	19	M	184.8	84.2	67.9	450
1022	Omega-3	21	M	177.4	84.2	57.4	325
1024	Placebo	21	M	180.6	71.1	67.1	375
1027	Placebo	21	M	178.7	72.2	69.6	375
1029	Omega-3	21	M	171.2	79.3	56.9	375
1033	Placebo	22	M	182.9	80.7	70.2	425
1034	Omega-3	22	M	189.5	87.1	59.8	475
1035	Placebo	19	M	186.8	86.9	67.6	400
1036	Placebo	20	M	183.6	76.3	67.2	400
1037	Placebo	26	M	175.1	72.1	61.4	300
Omega-3	Mean	20		177.6	78.87	60.2	379
	SD	1		7.7	7.29	4.5	70
Placebo	Mean	21		181.8	77.64	67.3	389
	SD	2		4.0	6.36	2.9	48

Appendix B: Erythrocyte Deformability Data

Subject	Supplement	BLPreN5Pa	BLPostN5Pa	BLPreH5Pa	BLPostH5Pa	BikePreN5Pa	BikePostN5Pa	BikePreH5Pa	BikePostH5Pa
1015	Omega-3	0.32033	0.324096	0.318463	0.318391	0.31353	0.321152	0.317627	0.32604
1017	Omega-3	0.310285	0.326433	0.315324	0.322035	0.313772	0.323711	0.322725	0.319975
1020	Omega-3	0.312107	0.329273	0.310985	0.324136	0.324713	0.327552	0.319583	0.327389
1021	Placebo	0.312229	0.318978	0.316848	0.316881	0.31279	0.322135	0.311651	0.299661
1022	Omega-3	0.326613	0.297534	0.318542	0.328869	0.318976	0.328795	0.308138	0.325348
1024	Placebo	0.332223	0.304511	0.318199	0.322163	0.318837	0.319554	0.319188	0.323978
1027	Placebo	0.322827	0.320403	0.300759	0.324286	0.316412	0.326754	0.309431	0.324098
1029	Omega-3	0.326953	0.240995	0.328522	0.314835	0.324722	0.311072	0.326974	0.327975
1033	Placebo	0.324320	0.330341	0.335229	0.325003	0.328549	0.318887	0.329256	0.328982
1034	Omega-3	0.330168	0.325654	0.328050	0.321189	0.33178	0.316018	0.322549	0.310807
1035	Placebo	0.326937	0.326869	0.316672	0.307477	0.329801	0.321489	0.315236	0.324724
1036	Placebo	0.328935	0.328511	0.315421	0.322400	0.332460	0.326468	0.325801	0.317048
1037	Placebo	0.324542	0.320021	0.321463	0.328993	0.313084	0.316754	0.332003	0.323619
Omega-3	Mean	0.321076	0.307331	0.319981	0.321576	0.321249	0.321383	0.319599	0.322922
	SD	0.008310	0.034524	0.007001	0.004810	0.007150	0.006835	0.006451	0.006581
Placebo	Mean	0.324573	0.321376	0.317799	0.321029	0.321705	0.321720	0.320367	0.320301
	SD	0.006306	0.008703	0.010129	0.006998	0.008348	0.003775	0.008824	0.009751

Subject	Supplement	BLPreN10Pa	BLPostN10Pa	BLPreH10Pa	BLPostH10Pa	BikePreN10Pa	BikePostN10Pa	BikePreH10Pa	BikePostH10Pa
1015	Omega-3	0.382316	0.386384	0.378467	0.380696	0.376125	0.383497	0.378366	0.389694
1017	Omega-3	0.374507	0.390634	0.376989	0.382908	0.376912	0.384793	0.384265	0.382266
1020	Omega-3	0.374365	0.398562	0.372994	0.387147	0.388844	0.395032	0.379715	0.394154
1021	Placebo	0.374001	0.381040	0.381296	0.378265	0.376216	0.383083	0.377816	0.367856
1022	Omega-3	0.391066	0.365668	0.381311	0.399992	0.380890	0.397577	0.374910	0.389450
1024	Placebo	0.406460	0.368480	0.381516	0.384901	0.382785	0.381550	0.383952	0.387772
1027	Placebo	0.386377	0.380519	0.367342	0.386243	0.380137	0.392247	0.372692	0.385940
1029	Omega-3	0.390866	0.347439	0.396642	0.376336	0.388041	0.375818	0.393451	0.395046
1033	Placebo	0.385958	0.402997	0.414189	0.388701	0.395129	0.380696	0.399450	0.398686
1034	Omega-3	0.402133	0.390671	0.395245	0.383539	0.406630	0.378870	0.381925	0.378233
1035	Placebo	0.392130	0.391136	0.377275	0.374813	0.399795	0.382986	0.376452	0.386988
1036	Placebo	0.397592	0.396912	0.375880	0.385040	0.407786	0.392365	0.389231	0.379601
1037	Placebo	0.388425	0.382144	0.385428	0.398196	0.379402	0.381189	0.407677	0.386935
Omega-3	Mean	0.385876	0.379893	0.383608	0.385103	0.386240	0.385931	0.382105	0.388141
	SD	0.010868	0.019379	0.009935	0.008117	0.011347	0.008693	0.006401	0.006643
Placebo	Mean	0.390135	0.386175	0.383275	0.385166	0.388750	0.384874	0.386753	0.384825
	SD	0.010173	0.011623	0.014787	0.007509	0.012106	0.005153	0.012889	0.009367

Subject	Supplement	BLPreN15Pa	BLPostN15Pa	BLPreH15Pa	BLPostH15Pa	BikePreN15Pa	BikePostN15Pa	BikePreH15Pa	BikePostH15Pa
1015	Omega-3	0.472282	0.476422	0.468041	0.470558	0.466412	0.474969	0.468255	0.481433
1017	Omega-3	0.466294	0.480595	0.466705	0.473329	0.467335	0.470330	0.474484	0.474185
1020	Omega-3	0.464102	0.484298	0.462106	0.477660	0.479560	0.485490	0.468997	0.484505
1021	Placebo	0.465405	0.472553	0.474006	0.467906	0.465869	0.472152	0.471555	0.460139
1022	Omega-3	0.480131	0.460206	0.472692	0.485416	0.471613	0.486225	0.467974	0.479114
1024	Placebo	0.489995	0.454688	0.474559	0.478570	0.474601	0.473932	0.474574	0.479777
1027	Placebo	0.477764	0.471521	0.459003	0.477253	0.470855	0.483356	0.463431	0.475158
1029	Omega-3	0.480383	0.436470	0.484867	0.467726	0.477949	0.466797	0.484152	0.486104
1033	Placebo	0.476697	0.489304	0.493695	0.480733	0.483114	0.472142	0.487482	0.485909
1034	Omega-3	0.490664	0.483004	0.478667	0.476673	0.493521	0.464191	0.470012	0.472056
1035	Placebo	0.480062	0.480825	0.467532	0.466276	0.486924	0.473150	0.465294	0.474284
1036	Placebo	0.486772	0.488903	0.464243	0.478094	0.492806	0.482267	0.478970	0.471477
1037	Placebo	0.480580	0.473126	0.481029	0.484639	0.472291	0.472765	0.495895	0.476007
Omega-3	Mean	0.475643	0.470166	0.472180	0.475227	0.476065	0.474667	0.472312	0.479566
	SD	0.010001	0.018693	0.008389	0.006221	0.010096	0.009395	0.006268	0.005589
Placebo	Mean	0.479611	0.475846	0.473438	0.476210	0.478066	0.475681	0.476743	0.474679
	SD	0.007895	0.011979	0.011528	0.006700	0.009724	0.004920	0.011740	0.007915

Subject	Supplement	BLPreN20Pa	BLPostN20Pa	BLPreH20Pa	BLPostH20Pa	BikePreN20Pa	BikePostN20Pa	BikePreH20Pa	BikePostH20Pa
1015	Omega-3	0.575022	0.576832	0.573359	0.573964	0.572481	0.579877	0.574090	0.583061
1017	Omega-3	0.574637	0.577653	0.571775	0.577343	0.572143	0.563409	0.576544	0.581391
1020	Omega-3	0.570315	0.568706	0.567947	0.578356	0.579018	0.580576	0.573281	0.579711
1021	Placebo	0.577168	0.580030	0.581317	0.572731	0.567670	0.573211	0.580435	0.564118
1022	Omega-3	0.574499	0.531696	0.578157	0.568235	0.577453	0.576324	0.571799	0.576338
1024	Placebo	0.567677	0.554158	0.584939	0.587779	0.579935	0.582784	0.576289	0.582695
1027	Placebo	0.580181	0.578644	0.564392	0.579622	0.575098	0.581142	0.572672	0.573999
1029	Omega-3	0.576596	0.544415	0.574730	0.577922	0.576486	0.571550	0.580850	0.582350
1033	Placebo	0.578397	0.572533	0.562994	0.582952	0.573550	0.579498	0.575403	0.572605
1034	Omega-3	0.577982	0.584447	0.560642	0.585216	0.576413	0.559396	0.569676	0.577951
1035	Placebo	0.572057	0.576967	0.575412	0.562719	0.573372	0.576405	0.569373	0.568701
1036	Placebo	0.577747	0.585133	0.568660	0.585311	0.572201	0.577419	0.576500	0.579268
1037	Placebo	0.582885	0.577878	0.593516	0.570413	0.578146	0.576292	0.580344	0.573265
Omega-3	Mean	0.574842	0.563958	0.571102	0.576839	0.575666	0.571855	0.574373	0.580134
	SD	0.002593	0.021064	0.006129	0.005589	0.002765	0.008798	0.003915	0.002623
Placebo	Mean	0.576587	0.575049	0.575890	0.577361	0.574282	0.578107	0.575859	0.573522
	SD	0.005123	0.009949	0.011351	0.009057	0.004020	0.003257	0.003960	0.006191

BL – Baseline
Pre – Pre-supplementation
H – Hypoxia
B – Bike (post- exercise)
Post – Post-supplementation
N – Normoxia
5/10/15/20Pa – Pascals of Sheer Stress

Appendix C: NIRS Data

Subject	Group	DN025S	DN025O	DN025D	DN025T	DN050S	DN050O	DN050D	DN050T
1015	Omega-3	-1.10	3.90	3.00	6.90	-4.50	3.90	7.60	11.40
1017	Omega-3	-0.40	0.80	0.50	1.30	-5.70	-3.80	2.50	-1.30
1020	Omega-3	-1.70	1.00	1.50	2.50	-4.60	-0.90	2.70	1.80
1021	Placebo	-2.00	-0.20	2.50	2.40	-6.00	-4.50	5.80	1.40
1022	Omega-3	-2.10	-1.40	2.10	0.80	-5.70	-4.00	3.50	-0.50
1024	Placebo	-2.40	0.10	3.40	3.40	-13.30	-10.50	14.70	4.10
1027	Placebo	-4.20	-1.30	5.30	4.00	-7.70	-3.00	9.80	6.70
1029	Omega-3	-1.40	0.40	1.60	2.20	-6.80	-2.80	6.70	4.10
1033	Placebo	-2.30	0.20	2.60	2.60	-10.40	-4.80	9.50	4.60
1034	Omega-3	-1.20	0.60	1.60	2.30	-6.30	-2.70	6.10	3.50
1035	Placebo	-2.60	2.40	4.80	7.20	-8.40	0.80	12.80	13.60
1036	Placebo	-5.00	-1.90	4.50	2.70	-9.20	-2.60	9.10	6.50
1037	Placebo	-1.80	1.20	2.10	3.30	-4.60	1.00	5.00	4.00
Omega-3	Mean								
	SD	-1.32	0.88	1.72	2.67	-5.60	-1.72	4.85	3.17
Placebo	Mean	0.58	1.71	0.82	2.18	0.91	2.96	2.21	4.56
	SD	-2.90	0.07	3.60	3.66	-8.51	-3.37	9.53	5.84

Subject	Group	DN075S	DN075O	DN075D	DN075T	DN0maxS	DN0maxO	DN0maxD	DN0maxT
1015	Omega-3	-6.90	4.90	11.50	16.40	-13.50	3.20	17.70	24.80
1017	Omega-3	-8.40	-3.30	4.50	1.20	-14.90	-7.30	7.40	0.00
1020	Omega-3	-5.00	-0.40	3.20	2.80	-13.90	-3.40	8.50	5.10
1021	Placebo	-6.40	-3.90	6.70	2.90	-13.10	-2.50	18.00	15.60
1022	Omega-3	-7.30	-4.20	4.50	0.30	-12.00	-5.80	7.20	1.40
1024	Placebo	-18.40	-13.90	21.40	7.30	-20.70	-14.20	25.90	11.50
1027	Placebo	-11.40	-4.00	15.20	11.20	-16.00	-8.70	20.10	11.40
1029	Omega-3	-11.80	-3.60	13.00	9.50	-15.20	1.40	21.50	23.00
1033	Placebo	-28.90	-18.30	25.90	7.50	-33.70	-20.90	31.80	10.80
1034	Omega-3	-9.40	-2.90	9.80	7.00	-14.20	-4.90	15.40	10.60
1035	Placebo	-9.50	-1.10	13.30	12.20	-12.30	-2.00	17.60	15.60
1036	Placebo	-14.90	-8.00	12.90	5.00	-16.30	-9.50	13.60	4.20
1037	Placebo	-6.10	-0.30	6.10	5.80	-7.10	0.10	7.40	7.40
Omega-3	Mean								
	SD	-8.13	-1.58	7.75	6.20	-13.95	-2.80	12.95	10.82
Placebo	Mean	2.33	3.44	4.19	6.11	1.14	4.19	6.09	10.79
	SD	-13.66	-7.07	14.50	7.41	-17.03	-8.24	19.20	10.93

Subject	Group	DN125S	DN125O	DN125D	DN125T	DN150S	DN150O	DN150D	DN150T
1015	Omega-3	-2.50	2.10	-17.30	5.70	-10.30	-5.90	9.70	3.70
1017	Omega-3	-2.40	0.30	1.70	2.00	-6.90	-2.20	3.80	1.60
1020	Omega-3	0.00	3.00	1.10	3.90	-6.40	-1.10	4.00	2.70
1021	Placebo	-0.40	3.20	1.70	4.80	-1.00	2.70	2.30	4.90
1022	Omega-3	-6.20	-1.50	3.60	2.10	-10.70	-4.00	5.90	1.90
1024	Placebo	-3.60	-2.20	4.10	1.90	-9.20	-6.40	10.40	4.00
1027	Placebo	-11.10	-9.50	11.40	1.90	-12.80	-8.80	14.80	6.00
1029	Omega-3	-3.70	1.00	5.20	6.20	-7.80	-1.80	-0.60	7.60
1033	Placebo	0.90	8.50	2.50	11.00	-7.00	5.60	15.00	20.50
1034	Omega-3	-0.50	3.40	2.30	5.60	-6.50	-0.20	9.90	9.70
1035	Placebo	-2.40	3.70	4.90	8.60	-6.00	4.50	10.20	14.80
1036	Placebo	-5.20	-0.60	6.10	5.60	-8.30	-3.40	8.80	5.40
1037	Placebo	-2.20	0.60	1.90	2.50	-3.80	1.40	3.60	5.00
Omega-3	Mean	-2.55	1.38	-0.57	4.25	-8.10	-2.53	5.45	4.53
	SD	2.25	1.83	8.33	1.87	1.93	2.08	3.99	3.34
Placebo	Mean	-3.43	0.53	4.66	5.19	-6.87	-0.63	9.30	8.66
	SD	3.93	5.63	3.39	3.53	3.81	5.60	4.94	6.39

Subject	Group	DN175S	DN175O	DN175D	DN175T	DN1maxS	DN1maxO	DN1maxD	DN1maxT
1015	Omega-3	-11.20	-5.30	11.20	5.80	-17.20	-9.30	17.30	7.90
1017	Omega-3	-5.80	-1.30	3.50	2.20	-8.40	-0.80	5.70	4.80
1020	Omega-3	-7.20	-1.80	4.40	2.40	-11.00	2.30	9.70	11.70
1021	Placebo	-2.70	0.90	3.60	4.50	-8.20	-3.90	8.20	4.30
1022	Omega-3	-11.60	-4.30	6.40	2.10	-17.50	-7.40	9.30	1.90
1024	Placebo	-12.00	-10.00	12.70	2.70	-19.40	-14.10	22.80	8.70
1027	Placebo	-15.80	-10.80	18.60	7.80	-17.00	-12.00	20.00	8.00
1029	Omega-3	-9.80	-2.40	12.00	9.60	-13.00	1.40	20.10	21.50
1033	Placebo	-12.80	2.50	24.90	27.50	-22.40	-4.80	43.10	38.40
1034	Omega-3	-13.70	-6.80	18.40	11.60	-19.50	-11.50	26.20	14.60
1035	Placebo	-9.40	2.10	13.60	15.70	-14.10	-0.50	19.40	18.90
1036	Placebo	-10.60	-5.00	11.10	6.10	-13.50	-7.40	13.90	6.50
1037	Placebo	-6.90	-0.30	5.50	5.10	-7.70	1.90	7.40	9.30
Omega-3	Mean	-9.88	-3.65	9.32	5.62	-14.43	-4.22	14.72	10.40
	SD	2.94	2.17	5.65	4.15	4.31	5.91	7.79	7.10
Placebo	Mean	-10.03	-2.94	12.86	9.91	-14.61	-5.83	19.26	13.44
	SD	4.26	5.67	7.32	8.82	5.47	5.80	12.06	11.93

Subject	Group	DH025S	DH025O	DH025D	DH025T	DH050S	DH050O	DH050D	DH050T
1015	Omega-3	-4.50	-3.20	3.60	0.50	-8.90	-4.20	8.40	4.30
1017	Omega-3	-3.60	-3.10	1.90	-1.30	-10.80	-6.30	6.60	0.30
1020	Omega-3	-3.10	-0.40	2.30	1.80	-6.00	-2.30	3.70	1.40
1021	Placebo	-3.60	-0.20	3.40	3.10	-5.40	-2.10	4.20	2.10
1022	Omega-3	-3.80	-1.20	2.00	0.90	-8.90	-3.50	4.60	1.20
1024	Placebo	-2.40	3.50	5.00	8.30	-11.50	-5.20	15.40	10.10
1027	Placebo	-6.60	-3.20	7.70	4.50	-13.40	-8.80	14.70	5.90
1029	Omega-3	-4.80	-0.70	5.60	4.90	-9.60	-1.80	12.00	10.10
1033	Placebo	-4.60	-2.00	6.10	4.10	-12.20	-7.90	15.90	8.10
1034	Omega-3	-6.70	-3.60	7.70	4.10	-13.20	-9.20	14.40	5.20
1035	Placebo	-4.70	-3.00	4.50	1.50	-9.30	-7.70	7.80	0.10
1036	Placebo	-6.00	-1.20	5.80	4.60	-13.80	-7.40	11.50	4.20
1037	Placebo	-4.00	-1.10	3.30	2.20	-6.60	-1.40	5.90	4.50
Omega-3	Mean	-4.42	-2.03	3.85	1.82	-9.57	-4.55	8.28	3.75
	SD	1.28	1.42	2.35	2.32	2.38	2.78	4.21	3.65
Placebo	Mean	-4.56	-1.03	5.11	4.04	-10.31	-5.79	10.77	5.00
	SD	1.42	2.26	1.57	2.21	3.31	2.97	4.82	3.41

Subject	Group	DH075S	DH075O	DH075D	DH075T	DH0maxS	DH0maxO	DH0maxD	DH0maxT
1015	Omega-3	-9.80	-0.30	12.00	11.70	-13.00	-1.50	15.90	14.40
1017	Omega-3	-10.70	-7.00	6.20	-0.80	-12.50	-8.10	7.20	-0.90
1020	Omega-3	-9.20	-4.80	5.10	0.20	-12.70	-6.10	7.20	1.10
1021	Placebo	-7.30	-3.30	5.50	2.10	-8.20	-2.70	6.80	4.10
1022	Omega-3	-11.80	-4.50	6.30	2.00	-12.90	-4.50	7.10	2.70
1024	Placebo	-15.20	-9.40	19.30	9.70	-17.30	-12.00	21.30	9.20
1027	Placebo	-13.20	-7.60	15.40	7.90	-15.00	-10.70	16.00	5.30
1029	Omega-3	-9.90	-0.80	13.20	12.40	-12.60	-2.60	16.40	13.80
1033	Placebo	-11.90	-16.00	9.00	-7.00	-15.80	-18.80	13.50	-5.30
1034	Omega-3	-14.70	-9.70	16.70	7.00	-16.80	-11.70	18.80	7.10
1035	Placebo	-12.40	-8.10	12.20	4.20	-11.00	-4.30	13.10	8.80
1036	Placebo	-13.60	-6.90	11.60	4.70	-13.50	-7.30	11.20	3.90
1037	Placebo	-6.30	1.40	6.90	8.30	-7.40	0.80	7.80	8.70
Omega-3	Mean	-11.02	-4.52	9.92	5.42	-13.42	-5.75	12.10	6.37
	SD	2.02	3.60	4.72	5.80	1.67	3.76	5.49	6.55
Placebo	Mean	-11.41	-7.13	11.41	4.27	-12.60	-7.86	12.81	4.96
	SD	3.33	5.36	4.83	5.64	3.83	6.58	4.94	5.07

Subject	Group	DH125S	DH125O	DH125D	DH125T	DH150S	DH150O	DH150D	DH150T
1015	Omega-3	-4.50	-0.40	5.70	5.20	-9.50	-4.80	10.70	5.80
1017	Omega-3	-4.00	-1.40	2.20	0.80	-7.10	-3.30	3.80	0.50
1020	Omega-3	-3.50	-0.20	2.10	2.00	-10.00	-5.40	4.00	-1.40
1021	Placebo	-2.00	1.20	2.40	3.60	-5.10	-1.50	4.00	2.50
1022	Omega-3	-3.70	-0.10	2.40	2.30	-7.40	-1.30	4.60	3.40
1024	Placebo	-6.50	-3.20	7.40	4.10	-12.80	-9.40	13.70	4.30
1027	Placebo	-11.10	-7.60	14.40	6.80	-20.90	-14.70	25.60	10.90
1029	Omega-3	-1.60	2.90	3.50	6.50	-5.50	1.00	8.40	9.30
1033	Placebo	-5.40	-1.10	8.20	7.20	-13.40	-7.80	19.50	11.60
1034	Omega-3	-3.60	-1.80	4.20	2.40	-12.10	-8.90	12.80	3.90
1035	Placebo	-4.60	-1.30	5.00	3.60	-9.60	-2.50	11.20	8.70
1036	Placebo	-1.90	0.80	2.60	3.40	-7.50	-3.60	7.00	3.40
1037	Placebo	0.60	-0.50	-0.80	-1.30	-8.00	-4.50	6.20	1.70
Omega-3	Mean	-3.48	-0.17	3.35	3.20	-8.60	-3.78	7.38	3.58
	SD	0.99	1.65	1.42	2.17	2.38	3.44	3.83	3.80
Placebo	Mean	-4.41	-1.67	5.60	3.91	-11.04	-6.29	12.46	6.16
	SD	3.82	2.99	4.97	2.79	5.24	4.66	7.81	4.14

Subject	Group	DH175S	DH175O	DH175D	DH175T	DH1maxS	DH1maxO	DH1maxD	DH1maxT
1015	Omega-3	-10.50	-3.10	13.20	10.00	-13.80	-4.30	18.00	13.60
1017	Omega-3	-9.80	-5.00	5.10	0.10	-11.50	-5.80	6.00	0.10
1020	Omega-3	-10.50	-5.80	4.10	-1.60	-15.90	-6.30	7.60	1.30
1021	Placebo	-5.80	-2.60	4.20	1.60	-3.70	0.00	3.40	3.40
1022	Omega-3	-11.20	-3.20	6.70	3.60	-11.60	-3.30	7.00	3.70
1024	Placebo	-15.10	-7.30	18.90	11.60	-19.90	-10.20	25.70	15.40
1027	Placebo	-30.30	-18.40	31.60	13.20	-25.70	-18.10	32.50	14.50
1029	Omega-3	-9.70	-1.90	13.40	11.60	-13.10	-0.40	20.80	20.50
1033	Placebo	-17.70	-12.40	25.20	12.70	-20.60	-14.30	30.50	16.20
1034	Omega-3	-15.30	-10.60	16.90	6.30	-16.00	-10.10	18.60	8.40
1035	Placebo	-12.70	-3.70	15.10	11.40	-12.90	-0.70	18.10	17.40
1036	Placebo	-11.20	-6.90	9.70	2.90	-14.60	-8.80	12.90	4.10
1037	Placebo	-6.80	-10.90	1.70	-9.20	-8.90	-11.20	3.40	-7.80
Omega-3	Mean	-11.17	-4.93	9.90	5.00	-13.65	-5.03	13.00	7.93
	SD	2.10	3.11	5.27	5.29	1.99	3.25	6.80	7.92
Placebo	Mean	-14.23	-8.89	15.20	6.31	-15.19	-9.04	18.07	9.03
	SD	8.25	5.47	10.93	8.33	7.52	6.67	12.10	9.41

D – Delta from 25% Unloaded	25 – Trial at 25% of Max Power	S – Tissue Saturation (%)
N – Normoxia	50 – Trial at 25% of Max Power	O – [OxyHB] (µm)
H – Hypoxia	75 – Trial at 25% of Max Power	D – [DeoxyHB] (µm)
0 – Pre-supplementation	Max – at Exhaustion	T – Total [HB+MB] (µm)
1 – Post-supplementation		

Appendix D: Informed Consent Form

INDIANA UNIVERSITY INFORMED CONSENT STATEMENT FOR

The effects of altered erythrocyte deformability on microvasculature oxygenation and exercise tolerance in adults with type 2 diabetes

You are invited to participate in a research study that will help determine the effects of omega-3 fatty acid (fish oil) supplementation on how you complete an exercise task. You were selected as a possible subject because you identified that you were either: a) a type 2 diabetic, b) an individual who does not regularly engage in training / physical activity, or c) a highly endurance trained individual. We ask that you read this form and ask any questions you may have before agreeing to be in the study.

Disclaimer: It is possible that you will not qualify for the study following the completion of the blood test, height, weight, or maximal exercise test.

The study is being conducted by Robert F. Chapman, Ph.D. (Principal Investigator), Timothy Mickleborough, PhD, Chad Wiggins, and Josh Foss in the Department of Kinesiology at Indiana University-Bloomington, and Kieren Mather, MD of the Department of Medicine at Indiana University. This study is funded by a 2013 American Diabetes Association Innovation Award.

STUDY PURPOSE

The purpose of the proposed study is to investigate how six weeks of omega-3 fatty acid (fish oil) supplements affects your ability to complete various exercise tasks.

NUMBER OF PEOPLE TAKING PART IN THE STUDY:

If you agree to participate, you will be one of 60 subjects who will be participating in this research.

PROCEDURES FOR THE STUDY:

If you agree to be in the study, the following items are included:

An invitation will be extended to visit the Human Performance laboratory a total of 6 times over a time period of approximately 10-12 weeks. Each visit will be done at a previously agreed-upon time. The first two visits last about 60 minutes and the remaining four visits last about 75 minutes. You should refrain from drinking caffeine for at least 8 hours prior to your visit. If you are an endurance trained athlete, you should refrain from exhaustive exercise (exercise that causes significant fatigue) for 24 hours prior to each visit. If you are an untrained individual (completing less than 90 minutes of physical activity a week), we ask that you maintain the same low level of physical activity throughout the study.

Visit #1
This visit includes a blood draw to measure your blood cholesterol, blood glucose, and hemoglobin values.

Visit #2

This visit includes the following tests: a) measures of your height, weight, resting pulse, and resting blood pressure, and b) a maximal exercise test on a stationary bicycle.

Visits #3 through #6
These visits include the following tests: a) a forearm handgrip test (i.e. repeated squeezing against a resistance), and b) a submaximal exercise test on a stationary bicycle. For these tests, you will either breathe room air or low oxygen air, simulating an altitude of approximately 9,900 feet, which is equivalent to an altitude you might experience in the Rocky Mountains.

Between Visits #4 and #5, you will be asked to take capsules that are omega-3 (fish oil) supplements or placebos (capsules of safflower oil that look like the fish oil supplement). You will take 8 capsules a day, 4 in the morning and 4 in the evening, each day for 6 weeks (42 consecutive days). You will be asked to record the date and time you take your capsules on a supplement diary sheet, which we will provide you. You may also record any comments you may have on this sheet. You will be asked to return the supplement diary sheet, the supplement containers, and any unused pills with your final visit to the lab (Visit #6).

Each of the tests and supplement routine is described below.

Blood draw (visit #1 only). A small amount of blood will be collected to measure your cholesterol, glucose, and hemoglobin levels. For this test, the inside of your arm, opposite your elbow will be swabbed with alcohol and a sterile needle will be briefly inserted. A small amount of blood (about 4 teaspoons) will be collected into a tube for analysis.

Height, weight, heart electrical activity, and blood pressure measures. Height will be measured by asking you to stand against a wall and a device will be lowered until it touches the top of your head. Weight will be measured by having you sit on a chair, which is placed on a scale. Blood pressure will be measured by placing a cuff around your upper arm. The cuff will be inflated, squeezing your upper arm, and quickly deflated. Adhesive electrodes will be placed on your chest and wires attached to monitor the electrical activity of your heart.

Maximal Cycle Exercise Test (visit #2 only). This exercise test will be completed on a stationary bicycle. Resting measurements will be collected for 5 minutes and followed by a 5 minute warm-up at a pace you select. The test begins with cycling at between 70 - 100 rpm with a light resistance load. Every minute, a small amount of additional resistance will be added until you can no longer maintain the required power output. The goal is for you to exercise for as long as you can, and for most individuals, this is about 8-12 minutes of pedaling.

Forearm Handgrip Test (visits #3 through #6 only). For this test, you will be asked to lie down on a padded table with your dominant arm (the arm you use to throw a ball) held out away from your side, resting on a table. You will be asked to wrap your fingers around a handgrip device, which is similar to a handle on a suitcase. You will be asked to squeeze the handle as hard as you can 3 times, resting between each squeeze. You will then be asked to perform 3 different exercise bouts of repeated squeezing. Repeated squeezing will be 5 seconds of squeezing and 5 seconds of relaxing. An audio recording will tell you when to squeeze and when to relax. The first two exercise bouts of repeated squeezing will be for 3 minutes, separated by 5 minutes of rest. The first bout will be at a low effort (17% of your maximal squeeze force) and the second at a medium effort (33% of maximal squeeze force). The third and last bout of repeated squeezing will be at 50% of your maximal squeeze force, done for as long as you can, or until your force falls to less than 33% of your maximal squeeze force for 3 consecutive squeezes. Normally, this third bout of repeated squeezing lasts about 4 to 7 minutes. You will be able to view a monitor that tells how hard you are squeezing and what target you are trying to achieve.

Submaximal Cycle Exercise Test (visits #3 through #6 only). This exercise test will be completed on a stationary bicycle. You will be asked to complete 3 separate cycle exercise efforts lasting 3 minutes each, separated by 10 minutes of seated rest. Each of the cycle exercise efforts will be at progressively higher resistances, equal to 25%, 50%, and 75%

of the peak resistance you achieved in the maximal cycle test. For each of the 3 cycle exercise efforts, resting measurements will be collected for 5 minutes and followed by a 5 minute warm-up at a very light resistance. You will be able to view a screen that shows the time completed.

All of the exercise tests (maximal cycle exercise, forearm handgrip, and submaximal cycle exercise) include wearing a clip that shines light through your index finger, either adhesive electrodes on your chest or a wireless heart rate monitor strap, and breathing either through a rubber mouthpiece while wearing nose clips, or a face mask which covers your nose and mouth. Either room air or low oxygen air simulating an altitude of approximately 9,900ft will flow into and out of your lungs as you breathe through the mouthpiece or face mask. You will not be told which air (room air or low oxygen air) you will be given. Rubber mouthpieces, face masks, heart rate monitor, and nose clips are cleansed in a detergent and antibacterial solution following each use. During each forearm exercise test, a fiber optic cable bundle will be secured by elastic bandages to your exercising forearm (about 1/3rd of the distance from your elbow to your wrist). During the submaximal cycle exercise test, a fiber optic cable bundle will be secured by elastic bandages to your left thigh and left calf. This device uses light waves to measure the oxygen content of your tissues.

Finger prick blood samples. Prior to the first exercise effort and immediately after each exercise effort of the forearm handgrip and submaximal cycle exercise test, we will prick your fingertip with a sterile needle and collect a few drops of blood. This means on each of Visits #3 through #6, you will receive a total of 8 finger pricks.

RISKS OF TAKING PART IN THE STUDY:

While on the study, the risks are:

Both maximal and moderate level exercise tests of healthy individuals, as described by the American College of Sports Medicine, presents little risk to the subject and does not require medical clearance for subjects under 40. For untrained and type 2 diabetic subjects, men over 45 years of age and women over 55 years of age, the risks associated with exercise testing increases. Potential risks and/or discomforts can include episodes of temporary light-headedness, chest discomfort, leg or arm cramps, occasional irregular heartbeats, and abnormal blood pressure responses. The risk of heart attack, although minor, (approximately 1 to 2 in 10,000) does exist, and a heart attack could result in death. During exercise testing you will be closely monitored for any abnormal changes in heart rate or breathing. You are free to indicate any discomfort and discontinue participation at any time.

There is a slight risk of skin discomfort or irritation from the fiber optic bundles and electrodes that will be placed on your skin.

There is a risk of blood pooling in your legs and low blood pressure immediately following the cycle exercise tests. After the tests, you will be allowed to cool down on the exercise bike. If at any time during testing you become light headed, you may have to lie down until you feel normal.

Risks associated with taking blood include excessive bleeding, fainting or feeling lightheaded, and possible infection. All blood samples will be taken by qualified individuals. A reasonable effort will be made to minimize the risks associated with drawing blood through the use of proper procedures and sterile techniques.

Potentially negative side effects could occur while taking the supplements. Common side effects include vomiting, nausea, bloating and burping. Rare side effects consist of easy bleeding/bruising and serious allergic reaction.

Breathing low oxygen air involves the risk of lightheadedness, heavy breathing, dry throat, and irritation of nasal passages due to the dry nature of the gas used.

In type 2 diabetic subjects, there is a risk of hypoglycemia (low blood sugar).

There is a potential risk of loss of confidentiality.

BENEFITS OF TAKING PART IN THE STUDY:

The benefits to participation that are reasonable to expect are information regarding your cholesterol and glucose levels, overall level of fitness, and how omega-3 fatty acid (fish oil) supplements may help you tolerate exercise better. Other than this information, you will gain little benefit. All subjects will be provided with feedback concerning their own results and the general findings of the study upon request.

CONFIDENTIALITY

Efforts will be made to keep your personal information confidential. Data will be stored on password protected computers in locked rooms with limited public access. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. Your identity will be held in confidence in reports in which the study may be published and databases in which results may be stored.

Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the study investigator and his/her research associates, the American Diabetes Association, the IU Institutional Review Board or its designees, and (as allowed by law) state or federal agencies, specifically the Office for Human Research Protections (OHRP) and the U.S. Food and Drug Administration (FDA), who may need to access the collected medical and/or research data.

PAYMENT

You will be paid a total of $150 for completing all (6) days of testing. Payment will be made by check and will be delivered by postal mail within approximately 4 weeks of your final testing session. If you withdraw prior to completing all days of testing, you will be paid according the exercise trial(s) you complete or attempt to complete; $30 per exercise trial (Visits 2-6).

COMPENSATION FOR INJURY

In the event of physical injury resulting from your participation in this research, necessary medical treatment will be provided to you at your own expense. Costs not covered by your health care insurer will be your responsibility. Also, it is your responsibility to determine the extent of your health care coverage. There is no program in place for other monetary compensation for such injuries. However, by signing this form you are not giving up any legal rights or benefits to which you are otherwise entitled.

CONTACTS FOR QUESTIONS OR PROBLEMS

For questions about the study or a research-related injury, contact the researcher Robert Chapman, Ph.D. at (812) 856-2452 or rfchapma@indiana.edu. If you cannot reach the researcher during regular business hours (i.e. 8:00AM-5:00PM), please call the IU Human Subjects Office at (812) 856-4242 or (800) 696-2949.

For questions about your rights as a research participant or to discuss problems, complaints or concerns about a research study, or to obtain information, or offer input, contact the IU Human Subjects Office at (812) 856-4242 or (800) 696-2949.

VOLUNTARY NATURE OF STUDY

Taking part in this study is voluntary. You may choose not to take part or may leave the study at any time. Leaving the study will not result in any penalty or loss of benefits to which you are entitled. Your decision whether or not to participate in this study will not affect your current or future relations with the investigators or Indiana University.

Your participation may be terminated by the investigator without regard to your consent in the following circumstances: blood pressure, ECG, cholesterol, height and weight, or maximal exercise test results that do not meet the criteria for inclusion in the study, an abnormal response to exercise testing, or an inability to complete the exercise tests.

SUBJECT'S CONSENT

In consideration of all of the above, I give my consent to participate in this research study.

I will be given a copy of this informed consent document to keep for my records. I agree to take part in this study.

Subject's Printed Name:_____

Subject's Signature:_____**Date**:_____

(must be dated by the subject)

Printed Name of Person Obtaining Consent:_____

Signature of Person Obtaining Consent:_____**Date**:_____

For IRB Office Use ONLY

IRB Approval Date:

Expiration Date:

Appendix E: Modified Physical Activity Readiness Questionnaire (PARQ)

Modified Physical Activity Readiness Questionnaire (PAR-Q)

Name			Date
DOB	**Age**	**Home Phone**	**Work Phone**

Regular exercise is associated with many health benefits, yet any change of activity may increase the risk of injury. Please read each question carefully and answer every question honestly:

Yes	**No**	**1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
Yes	**No**	**2. Do you feel pain in your chest when you do physical activity?**
Yes	**No**	3. **In the past month, have you had chest pain when you were not doing physical activity?**
Yes	**No**	**4. Do you lose your balance because of dizziness or do you ever lose consciousness?**
Yes	**No**	**5. Do you have a bone or joint problem that could be made worse by a change in your physical activity?**
Yes	**No**	**6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
Yes	**No**	**7. Do you know of any other reason you should not do physical activity?**
Yes	**No**	**8. Has your doctor ever told you that you have diabetes?**
Yes	**No**	**9. Has your doctor ever told you that you have high blood pressure?**
Yes	**No**	**10. Has your doctor ever told you that you have high cholesterol?**
Yes	**No**	**11. Has your doctor ever told you that you have high blood sugar?**
Yes	**No**	**12. Do you smoke?**
Yes	**No**	**13. Are you currently inactive?**
Yes	**No**	**14. Do you have a father, brother or son with heart disease before the age of 55 years old or a mother, sister or daughter with heart disease before the age of 65 years old?**

15. Measure height and weight to determine BMI:
Height:_____
Weight:_____

16. Measure resting BP and pulse
BP:_____ Pulse:_____

Participant Signature	Date

Appendix F: General Study Questionnaire

General Study Questionnaire

Name	**Date**

On average over the last 8 weeks, how many minutes per week did you exercise?	
Do you consider yourself to be a highly endurance trained individual?	**(Circle one) YES NO**

Participant Signature	**Date**

Appendix G: Omega-3 Food Frequency Questionnaire

Omega-3 Food Frequency Questionnaire

Department of Kinesiology

Human Performance Laboratory

Indiana University

Food Frequency Questionnaire from: Hanson JA, Lin Y, Strandjord SE, and Hibbeln JR. The Relationship between Omega-3 HUFA Score and Dietary Intake of EPA and DHA among U.S. Soldiers. *Journal of the Academy of Nutrition and Dietetics* 113: A26, 2013

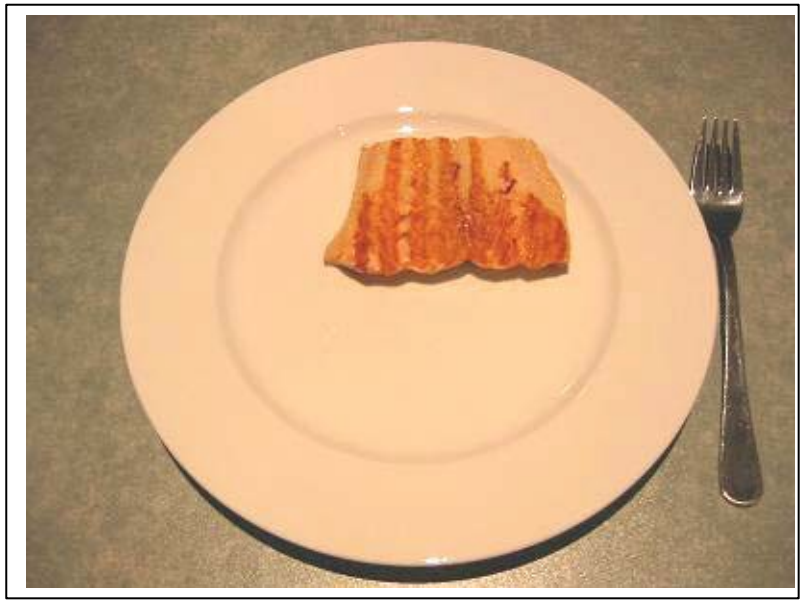
Instructions:

This questionnaire is about how much and how often you ate foods containing high levels of omega-3 fatty acids. It is also about how much and how often you consume different beverages. When answering, think about what you usually ate and drank during the last six months. Please remember to include foods you ate in restaurants, as takeout food, and fish you or someone you know caught. Complete **PARTS 1-3**.

Part 1 Instructions:
Step 1: Mark the column with an "X" to show how often on average you ate the food.
Step 2: Mark your usual serving size with an "X", as small (S), medium (M), or large (L).

Please note:

- A small serving is about one-half (1/2) the medium serving size, or less.
- A large serving is about one-and-a half (1 ½) times the medium serving size or more.
- If you never ate a food, mark "never" and omit the serving size.
- Please do not skip any foods or leave blanks.
- 4 ounces of cooked fish looks like the picture on the right :



Example: This person never ate sushi, but ate a medium serving of baked or broiled white fish once a week.

	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2 + per day	Medium Serving size	S	M	L
Sushi	X									1 roll			
Baked or broiled white fish (such as snapper, cod, halibut, sole)				X						4 ounces		X	

Food Frequency Questionnaire from: Hanson JA, Lin Y, Strandjord SE, and Hibbeln JR. The Relationship between Omega-3 HUFA Score and Dietary Intake of EPA and DHA among U.S. Soldiers. *Journal of the Academy of Nutrition and Dietetics* 113: A26, 2013

PART 1

How often did you eat these foods during the last 6 months?

	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2 + per day	Medium Serving size	S	M	L
Canned tuna, tuna salad, tuna sandwich, tuna casserole										1 can or 1 cup casserole			
Fried fish, fish sandwich, fish sticks										4 ounces or 1 sand-witch			
Shellfish (shrimp, clams, oysters, lobster, crayfish)										4 ounces			
Sardines										1 can			
Baked, broiled, or grilled white fish (such as snapper, cod, halibut, sole)										4 ounces			
Baked, broiled, or grilled dark or oily fish (such as salmon, mackerel, and bluefish)										4 ounces			
Sushi Please write type _____										2 rolls			
Beef										4 ounces			
Pork										4 ounces			
Dark chicken meat										2 pieces			
Eggs with yolks										2 egg			

Food Frequency Questionnaire from: Hanson JA, Lin Y, Strandjord SE, and Hibbeln JR. The Relationship between Omega-3 HUFA Score and Dietary Intake of EPA and DHA among U.S. Soldiers. *Journal of the Academy of Nutrition and Dietetics* 113: A26, 2013

PART 2

See the display for examples of foods enriched with DHA and/or other omega-3 fatty acids. Did you eat any DHA or omega-3 enriched foods during the last 6 months? (Circle) **YES** or **NO**

If NO skip to Part 3. If YES, write in: 1) the food, 2) your usual serving size, and 3) how often eaten.

Food	Usual Serving Size	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2 + per day
Example : Horizon DHA milk	8 oz			X						

Food Frequency Questionnaire from: Hanson JA, Lin Y, Strandjord SE, and Hibbeln JR. The Relationship between Omega-3 HUFA Score and Dietary Intake of EPA and DHA among U.S. Soldiers. *Journal of the Academy of Nutrition and Dietetics* 113: A26, 2013

PART 3

Did you take any nutritional supplements during the last 6 months? (Circle) **Yes** or **No**
If NO, skip to end. If YES, how much and how often?

Supplement	How much did you normally take?
Cod liver oil, fish oil, or omega -3 supplements Please Specify 1._____ 2._____	 1._____ 2._____
Multivitamin Please Specify 1._____ 2._____	 1._____ 2._____
Other vitamin, mineral or nutritional supplements Please Specify 1._____ 2._____	 1._____ 2._____

END

Food Frequency Questionnaire from: Hanson JA, Lin Y, Strandjord SE, and Hibbeln JR. The Relationship between Omega-3 HUFA Score and Dietary Intake of EPA and DHA among U.S. Soldiers. *Journal of the Academy of Nutrition and Dietetics* 113: A26, 2013